

FORUM REVIEW ARTICLE

The Roles of Mitochondrial Damage-Associated Molecular Patterns in Diseases

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Abstract

Significance: Mitochondria, vital cellular power plants to generate energy, are involved in immune responses. Mitochondrial damage-associated molecular patterns (DAMPs) are molecules that are released from mitochondria to extracellular space during cell death and include not only proteins but also DNA or lipids. Mitochondrial DAMPs induce inflammatory responses and are critically involved in the pathogenesis of various diseases. **Recent Advances:** Recent studies elucidate the molecular mechanisms by which mitochondrial DAMPs are released and initiate immune responses by use of genetically modulated cells or animals. Importantly, the levels of mitochondrial DAMPs in patients are often associated with severity and prognosis of human diseases, such as infection, asthma, ischemic heart disease, and cancer. **Critical Issues:** Although mitochondrial DAMPs can represent proinflammatory molecules in various experimental models, their roles in human diseases may be multifunctional and complex. It remains unclear where and how mitochondrial DAMPs are liberated into extracellular spaces and exert their biological functions particularly *in vivo*. In addition, while mitochondria can secrete several types of DAMPs during cell death, the interaction of each mitochondrial DAMP (*e.g.*, synergistic effects) remains unclear. **Future Directions:** Regulation of mitochondrial DAMP-mediated immune responses may be important to alter the progression of human diseases. In addition, measuring mitochondrial DAMPs in patients may be clinically useful as biomarkers to predict prognosis or response to therapies. Further studies of the mechanisms by which mitochondrial DAMPs impact the initiation and progression of diseases may lead to the development of therapeutics specifically targeting this pathway. *Antioxid. Redox Signal.* 23, 1329–1350.

Introduction

FOR THE LAST decades, mitochondria have been extensively studied as critical cellular organelles for energy generation, protein synthesis, catabolism, and cell death (135, 158). Mitochondria are thought to be evolved from an endosymbiont α -proteobacterium and uniquely have their own DNA, which is duplicated during mitochondrial division (43). Recent studies reveal that mitochondria are diversely associated with immune responses (5, 158) and diseases (135, 138, 158). When mitochondria are damaged, the dysfunctional mitochondria increase generation of mitochondrial reactive oxygen species (ROS) in cells (107, 182). These dysfunctional mitochondria are prone to enhance immune responses (107, 142). In addition, recent reports suggest that

various mitochondrial molecules can be translocated to the outsides of mitochondria (*e.g.*, cytosol, cell surface, or extracellular spaces) and promote immune responses.

Against invading pathogens, cells exert their defense system by secretion of inflammatory cytokines/chemokines, activating the adaptive immune system and promoting phagocytosis (2). These immune responses are initiated by recognition of pathogen-associated molecular patterns (PAMPs) through both the plasma membrane receptors and the intracellular receptors such as toll-like receptors (TLRs) or nucleotide-binding and oligomerization domain (NOD)-like receptors (2, 30, 125, 136). Each receptor distinctively recognizes various microbe-associated components. These receptor molecules can also activate immune function in response to nonmicrobe-associated molecules (21). When cells are injured or dying by

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mechanical stress, microbial infection, or other various environmental stresses, these cells release their own components to the extracellular space called damage-associated molecular patterns (DAMPs) (21, 140). DAMPs include DNA, high-mobility group box 1 (HMGB1), or heat shock proteins (21, 140). Similar to PAMPs, these host-derived molecules can be recognized by the receptors, including TLRs or NOD-like receptors (NLRs), and trigger immune responses in various immune cells (*e.g.*, macrophages, dendritic cells [DCs], neutrophils) (21, 140). While original sources of DAMPs include nuclear, plasma membrane, and intracellular proteins (49), recent reports suggest that mitochondria are also major sources of DAMPs (179). The roles of isolated mitochondria on immune responses have been studied before the concept of mitochondrial DAMPs was proposed. For example, in 1982, Carp reported that human mitochondria disrupted by detergent or sonication display chemotaxis of polymorphonuclear leukocyte (PMN) (19). It is also reported that condition media harvested from necrotic cells contain mitochondrial components such as *N*-formyl peptides (NFPs) and trigger chemotaxis of platelets (28). Although the concise roles of mitochondria on inflammatory response and tissue injuries need to be further elucidated (10, 13, 178, 179), there are a number of reports showing that mitochondria-associated molecules exert various

pathophysiological functions. Mitochondria can release the mitochondria-associated molecules when cells are dying in response to the cellular stress, including mechanical stress or infection (Fig. 1). These molecules are called mitochondrial DAMPs and show various immune responses on immune cells such as macrophages or neutrophils (Fig. 1 and Table 1). Although the studies of DAMPs have been focusing on the molecules released to extracellular compartments, recent data suggest that mitochondrial DAMPs (particularly mitochondrial DNA [mtDNA]) released from mitochondria initiate immune responses in the cytosol (107, 131, 142, 163, 164). In addition, the molecular mechanisms by which cells control the escape of mitochondrial DAMPs also have been suggested (*e.g.*, autophagy-related proteins or deoxyribonuclease II [DNase II]) (107, 111). Since the impairment of these molecules increases inflammatory responses and susceptibility to the oxidative stress *in vivo* (107, 111), the roles of mitochondrial DAMPs in the subcellular compartment are likely to be critical in the pathogenesis of human diseases.

As shown in Table 1, mitochondrial DAMPs include various types of mitochondrial molecules and are released into extracellular spaces or cytosolic spaces. In addition, the functional roles of mitochondrial DAMPs are observed in various disease models both *in vitro* and *in vivo* (Table 1 and Fig. 2). Thus,

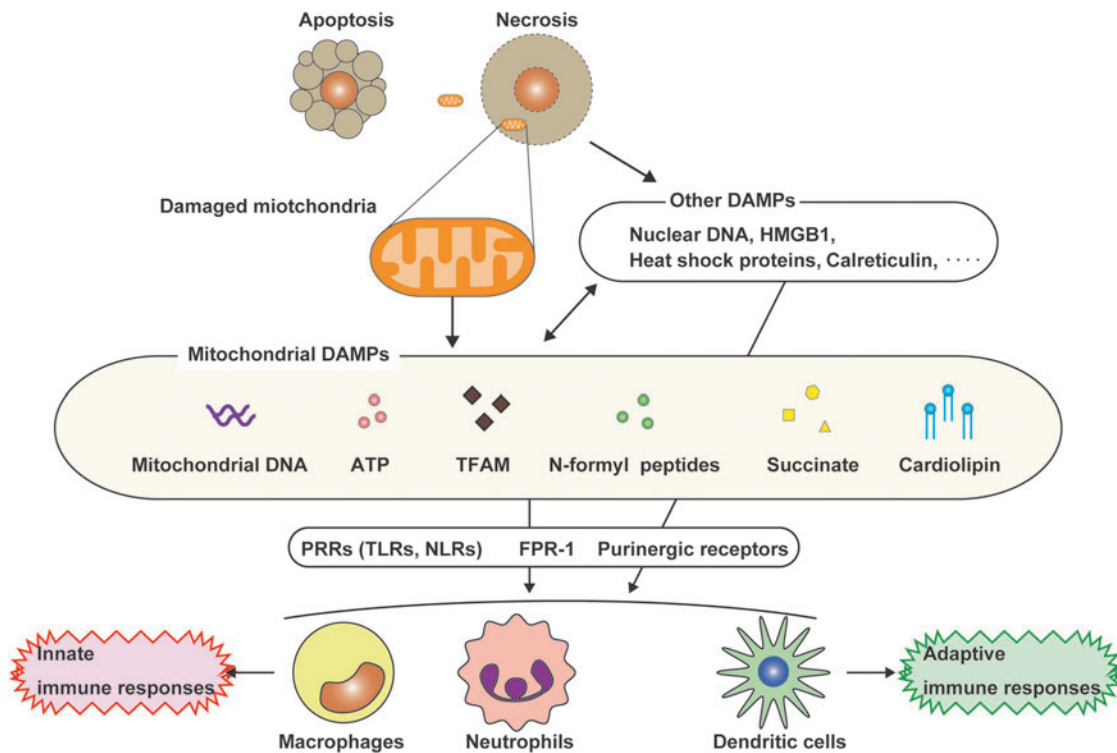


FIG. 1. DAMPs released from mitochondria. During cell death, various cellular components are released to the extracellular space or exposed to cell surface. The molecules released from dying cells have immune functions and are involved in inflammatory response. These molecules are called DAMPs. Of note, mitochondria release various mitochondrial components, which are involved in inflammatory responses (mitochondrial DAMPs). Damaged cells caused by trauma or infection may undergo cell death, including necrosis. Under these conditions, the necrotic cells can massively leak intracellular components, including mitochondria-related molecules, whereas it is believed that the release of DAMPs from apoptotic cells is limited. Under these conditions, the cells can leak intracellular components, including mitochondria-related molecules. The released or exposed mitochondrial DAMPs can initiate innate or adaptive immune responses by activating cell surface receptors (*e.g.*, P2X7R or FPRs) or intracellular receptors (*e.g.*, TLR9 or NLRP3) after their internalization into the cells. DAMPs, damage-associated molecular patterns; FPR, formyl peptide receptor; TLR, toll-like receptor. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

TABLE 1. MITOCHONDRIA-DERIVED DAMAGE-ASSOCIATED MOLECULAR PATTERNS

Mitochondrial DAMPs	Models releasing mitochondrial DAMPs into cytosol, cell surface, or extracellular space (in vitro and in vivo) (Ref.)	The roles as mitochondrial DAMPs (Ref.)	The roles of mitochondrial DAMPs in animal disease models (Ref.)
mtDNA	<p>Cytosol: ATP treatment in TLR ligand-primed macrophages (70, 106, 107, 142).</p> <p>Cytosol: Activation of Bak and Bax (131, 164).</p> <p>Cytosol: TFAM depletion (163).</p> <p>Media: Treatment with nitric oxide donor or PMA in neutrophils (75).</p> <p>Media: Treatment with LPS and IL-5 or IL-5 and C5a in eosinophils (171).</p> <p>Media: Treatment with IgE/anti-IgE in mast cells (176).</p> <p>Media: LPS or C5a treatment in GM-CSF-primed neutrophils (172).</p> <p>Serum or brain (cytosol): Irradiated mice (41).</p> <p>Plasma: Trauma/hemorrhagic shock in rats (178).</p> <p>Plasma: Bacillus <i>anthracis</i> infection in baboons (155).</p> <p>Serum: Acetaminophen-induced acute liver injury in mice (97).</p> <p>Media: Treatment with recombinant sPLA2-IIA in human platelet (10).</p>	<p>AIM2 inflammasome activation (107).</p> <p>NLRP3 inflammasome activation (107, 142).</p> <p>Adhesion of neutrophils and endothelial cells (153).</p> <p>Activation of TLR9 and NF-κB.</p> <p>Secretion of proinflammatory cytokine/chemokine (128, 177, 179).</p> <p>Secretion of proteases (e.g., MMP-8 and -9) (178).</p> <p>Production of type I INF through the cGAS/STING pathway (131, 163, 164).</p>	<p>IL-5 transgenic mice display intestinal eosinophil infiltration and extracellular mtDNA deposition in response to CLP-induced polymicrobial sepsis (171).</p> <p>Intravenous injection of disrupted mitochondria induces acute lung injury and inflammatory responses in rats (59, 179).</p> <p>Intravenous injection of mtDNA induces inflammation and lung injury in rats (177).</p> <p>Intra-articular injection of mtDNA induces arthritis in mice (25).</p> <p>Activated protein C treatment reduces plasma level of mtDNA and lethality in response to <i>Bacillus anthracis</i>-induced sepsis in baboons (155).</p> <p>Cardiac-specific deletion of DNase IIa increases the mortality and causes severe myocarditis with accumulation of mtDNA deposition in autolysosome in the myocardium of mice with cardiac hypertrophy (111).</p> <p>Deletion of LOX-1 shows arteriosclerosis with accumulation of mtDNA and inflammation in aorta of the mice (36).</p> <p>Acetaminophen treatment induces marked systemic inflammation and lung injury in mice, which is prevented in TLR9-deficient mice (97).</p>

(continued)

TABLE 1 (CONTINUED)

Mitochondrial DAMPs	Models releasing mitochondrial DAMPs into cytosol, cell surface, or extracellular space (in vitro and in vivo) (Ref.)	The roles as mitochondrial DAMPs (Ref.)	The roles of mitochondrial DAMPs in animal disease models (Ref.)
ATP	<p>Media: Fungal exposure (<i>Alternaria alternate</i>) in human bronchial epithelial cells (79).</p> <p>Media: Coculture with epithelial HaCat cells (an immortalized human keratinocyte line) and allogeneic PBMCs or buccal epithelial cells and autologous PBMCs (165).</p> <p>Media: Coculture of blood clots and primary retinal cells (109).</p> <p>Media: IgE and anti-IgE in mast cells (176).</p> <p>Media: NFP treatment in neutrophils (22).</p> <p>Media: MTX treatment in CT26 colorectal carcinoma cells (101).</p> <p>Media: Bleomycin (BLM) treatment in BEAS2B or MLE12 lung epithelial cells (129).</p> <p>BALF: Mice sensitized by i.p. injection of OVA (65).</p> <p>BALF: BLM-injected mice (129).</p> <p>Peritoneal fluid: Irradiated mice (165).</p>	<p>Potassium efflux through P2XR or P2YR (71, 79).</p> <p>Calcium mobilization (8, 106)</p> <p>NLRP3 inflammasome activation and release of mtDNA to cytosol (106, 107, 142).</p> <p>Mitochondrial ROS generation and dysfunction (106, 107, 142).</p> <p>NADPH oxidase-dependent ROS generation (61, 105).</p> <p>Activation of MAPK pathways (116, 143).</p> <p>Fusion of phagosome and lysosome (promoting killing of intracellular pathogens) (42).</p> <p>Chemotaxis (<i>e.g.</i>, neutrophils) (22, 63, 166).</p> <p>Cell death (69, 149, 181)</p>	<p>Administration of apyrase (ATP-diphosphatase) or suramin inhibits eosinophilic airway inflammation, Th2 cytokine production, and bronchial hyper-reactivity in mice sensitized by the injection of OVA with alum (65).</p> <p>Treatment of suramin and oxidized ATP (antagonists of P2receptor) or genetic deletion of P2X7R inhibits IL-33 release and Th2 responses in fungus-induced airway inflammation in mice (79).</p> <p>Neutralization of ATP by apyrase and genetic deficiency of P2X7R during irradiation-induced GVHD development improve survival of the mice. Stimulation of APCs with ATP promotes IFN-γ production and donor T-cell expansion (165).</p> <p>Deficiency of P2Y2R suppresses recruitment of neutrophils in the peritoneal cavity of mice after intraperitoneal injection of <i>Staphylococcus</i> bacteria (22).</p> <p>Administration of suramin and apyrase inhibits BLM-induced lung inflammation in mice, while injection of ATP further enhances the lung inflammation. Treatment of Pannexin-1 mimetic inhibitory peptide or genetic deletion of P2X7R inhibits bleomycin-induced lung inflammation and fibrosis in mice (129).</p> <p>Subcutaneous injection of MTX-treated cancer cells induces antitumor effects <i>in vivo</i>, which are neutralized by addition of oxidized ATP or suramin (101).</p>
TFAM	<p>Serum: Hemorrhagic shock in rats (20).</p>	<p>Triggering secretion of proinflammatory cytokines (<i>e.g.</i>, TNF and IL-6) in macrophage (20).</p> <p>Promoting secretion of proinflammatory cytokine by costimulation with CpG DNA or NFP (27, 68).</p>	<p>Deletion of TFAM in mice displays severe mtDNA depletion and embryonic lethal (86).</p> <p>Administration of recombinant TFAM increases the level of IL-6 and TNF and lactate in serum and MPO activity in the lungs of rats (20).</p>

(continued)

TABLE 1 (CONTINUED)

Mitochondrial DAMPs	Models releasing mitochondrial DAMPs into cytosol, cell surface, or extracellular space (in vitro and in vivo) (Ref.)	The roles as mitochondrial DAMPs (Ref.)	The roles of mitochondrial DAMPs in animal disease models (Ref.)
NFP	Media: Hypoxia-induced necrosis in platelets (28).	Calcium influx (179). Promoting secretion of IL-8 by costimulation with mtDNA or CpG (179). Secretion of proteases (MMP-8) (179). Chemotaxis of neutrophils or platelets through FPR1 (19, 28, 179).	Intravenous injection of disrupted mitochondria induces acute inflammatory lung injury in rats (179). FPR1 ^{-/-} mice increase susceptibility with <i>Listeria monocytogenes</i> infection (48). Acetaminophen-treated mice exhibit marked systemic inflammation and lung injury, which is prevented by CXCR2-FPR1 blockage (97).
Cardiolipin	Cell surface: Fas ligand-induced cell death in U937 (148).	NLRP3 inflammasome activation (66). A potent surfactant inhibitor (127). Binding to Atp8b1 (127). Binding to CD1d and stimulating CD1d-restricted $\gamma\delta$ T cells (35). Cell death (cytochrome c) (113).	Mice given intratracheal injection of cardiolipin display significantly lower lung compliance and higher elastance and resistance compared with the controls (127). Atp8b1 binds and internalizes cardiolipin from extracellular fluid to lung epithelia. Atp8b1 mutant mice display higher levels of cardiolipin in BALF and abrogate bacteria-induced acute lung injuries. Administration of a peptide, including the cardiolipin-binding motif or Atp8b1 gene transfer in mice, lessens bacteria-induced lung injury and improves survival (127).
Succinate	Media: Antimycin treatment in myoblasts (141).	Intracellular calcium mobilization (132). Chemotaxis and cytokine production in DC through GPR91 (132). Antigen-specific activation of helper T cells through the G protein-coupled receptor GPR91 of DCs (132). IL-1 β production in macrophages by stabilizing HIF-1 α (157).	Knockout of succinate receptor GRP91 improves survival of skin allograft in mice (132). Succinate treatment further increases the expression of GRP91 and aggravates right ventricular hypertrophy in rats with pulmonary artery banding (168).

AIM2, absent in melanoma 2; APC, antigen-presenting cells; ATP, adenosine triphosphate; BALF, bronchial alveolar lavage fluid; cGAS, cyclic GMP-AMP synthase; CLP, cecal ligation and puncture; DAMPs, damage-associated molecular patterns; DC, dendritic cell; DNase IIa, deoxyribonuclease IIa; FPR1, formyl peptide receptor 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; GVHD, graft-versus-host disease; HIF-1 α , hypoxia-inducible factor 1- α ; IFN- γ , interferon- γ ; IgE, immunoglobulin E; IL, interleukin; LOX-1, lectin-like, oxidized low-density lipoprotein receptor-1; LPS, lipopolysaccharide; Media, cell culture media; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MPO, myeloperoxidase; mtDNA, mitochondrial DNA; MTX, mitoxantrone; NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase; NFP, N-formyl peptides; OVA, ovalbumin; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; STING, stimulator of interferon genes; TFAM, mitochondrial transcription factor A; Th2, T helper 2; TLRs, toll-like receptors; TNF, tumor necrosis factor.

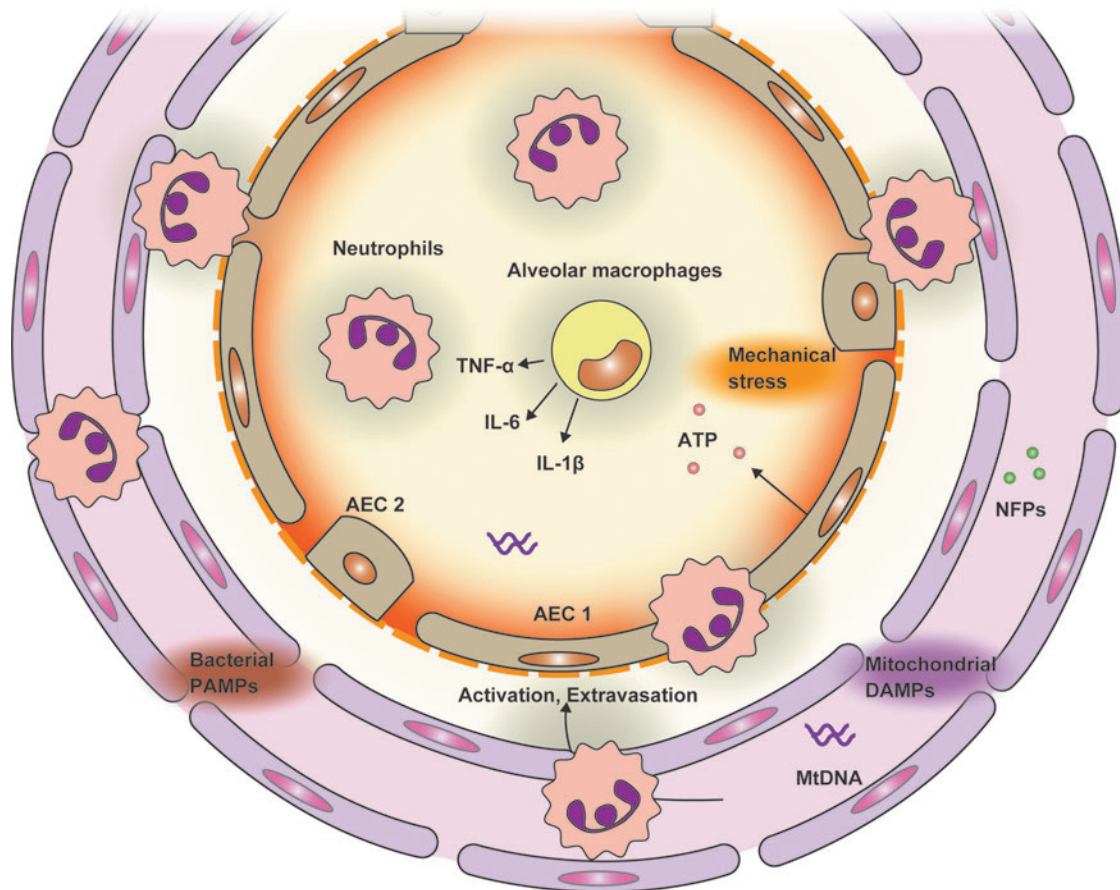


FIG. 2. The roles of mitochondrial DAMPs on organ injuries. Circulating mitochondrial DAMPs can induce peripheral distant organ injuries. After mitochondrial DAMPs are released into circulation from damaged tissue sites, mitochondrial DAMPs promote adhesion of activated neutrophils and vascular endothelial cells and transmigration of immune cells into distant organs such as the lung. The transmigrated immune cells secrete proinflammatory cytokines and proteinases, leading to inflammatory responses in alveolar spaces. In addition, this immune response may release another mitochondrial DAMP (e.g., ATP) and further enhance inflammatory responses, resulting in exacerbating lung injuries. ATP, adenosine triphosphate. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

besides the well-known roles of mitochondria as energy generators, mitochondria are DAMP-enriched cellular storage spaces where various cellular stresses can release mitochondrial components with their new biological roles on immune systems.

In this study, we review the functional roles of mitochondrial DAMPs in the various disease models. We will also discuss the association between mitochondrial DAMPs and human diseases.

Mitochondrial DAMPs

Mitochondrial DNA

MtDNA encodes 37 genes and all of them are essential for normal mitochondrial function (43). The association between mutation of mtDNA and human diseases is well characterized and studied (135, 158). However, it has been unclear if mtDNA has its own direct biological activities, including immune responses. The roles of mtDNA in immune systems

and its molecular mechanisms were elucidated from the studies of bacterial DNA (2, 112). TLR9 is a member of the TLR family and is expressed in various types of cells, especially immune cells. TLR9 is localized within the intracellular endosomal compartment and recognizes unmethylated CpG sequences in DNA molecules, which are abundant in bacterial genome and virus DNA (2, 80, 112). Upon recognition, TLR9 initiates immune response, including production of proinflammatory cytokine, chemotaxis, and phagocytosis, through myeloid differentiation primary response gene 88 (MyD88)-dependent pathways (2, 80). Thus, TLR9 exerts various immune responses by binding the unmethylated CpG site of microbial DNA. Mitochondria evolve from saprophytic bacteria to endosymbionts to organelles; therefore, mtDNA contains CpG DNA repeats and is mostly unmethylated (31, 51, 54, 133, 156). Stimulation with mtDNA can increase TLR9 expression in macrophages (177), and mtDNA-mediated activation of p38 MAPK is blocked by cotreatment of inhibitory

oligodeoxynucleotides (TTAGGG) that bind CpG motifs (179). These suggest that endogenous host mtDNA can be an inside self-activator of immune systems.

The roles of mtDNA in diseases have been studied by exogenous treatment of mtDNA or mice deficient in mtDNA receptors or binding molecules (Table 1). For example, the roles of mtDNA on inflammatory responses through TLR9 are studied in mice lacking DNase II, an acid DNase localized in the lysosome (36, 111). DNase II has an essential role in the degradation of the DNA of apoptotic cells after macrophages engulf them (64, 73, 74). Cardiac-specific deletion of lysosomal DNase II shows increased mortality and causes severe myocarditis and dilated cardiomyopathy after treatment with pressure overload (111). In addition, DNase II-deficient hearts display infiltration of inflammatory cells, increased gene expression of proinflammatory cytokines, and accumulation of mtDNA deposits in autolysosomes (111). Furthermore, administration of the inhibitory oligodeoxynucleotides against TLR9 or deletion of *tlr9* attenuated the development of cardiomyopathy in DNase II-deficient mice (111). Similar roles of mtDNA on inflammatory responses and tissue injuries are also observed in lectin-like, oxidized low-density lipoprotein receptor-1 (LOX-1)-deficient mice, a model of atherosclerosis (36). Accumulated damaged mtDNA due to deficiency of DNase II activates TLR9, leading to the development of atherosclerosis (36). Acetaminophen treatment increases the levels of circulating mtDNA and liver dysfunction in mice, and TLR9-deficient mice display decreased neutrophil-mediated

inflammatory responses and liver injury in the acetaminophen-treated mice (97). Recent studies show that extracellular mtDNA activates not only neutrophils (178, 179) but also vascular endothelial cells (153). The activation of these cells by mtDNA promotes adhesion of neutrophils to the endothelium and transmigration of the immune cells, leading to distant organ inflammatory responses (Fig. 3A). Importantly, injection of mtDNA induces acute lung injury (177) and arthritis with infiltration of mononuclear cells in mice (25), suggesting the direct roles of mtDNA on inflammation and tissue injuries *in vivo*. Furthermore, fragmented mtDNA containing CpG motif spontaneously induces plasmacytoid DC activation (128). These data suggest mtDNA is critically involved in the inflammatory responses and tissue injuries of animal disease models through TLR9 signaling pathways.

Another important mechanism by which mtDNA triggers immune responses is the inflammasome. Inflammasomes are multiprotein complexes that activate caspase-1 and downstream immune responses, including the maturation and secretion of proinflammatory cytokine (30, 125, 136). The inflammasomes contain a member of the NLR family (Nod and leucine-rich repeat-containing) or PYHIN family (pyrin domain [PYD] and HIN domain-containing) (30, 125). NLR proteins are key mediators of the inflammasome as studies of gene-deficient mice and cells show that NLR inflammasomes are critically implicated in host defense, cancers, and metabolic and autoimmune disorders (30, 137). Among identified inflammasomes, the NLRP3 inflammasome is the most studied

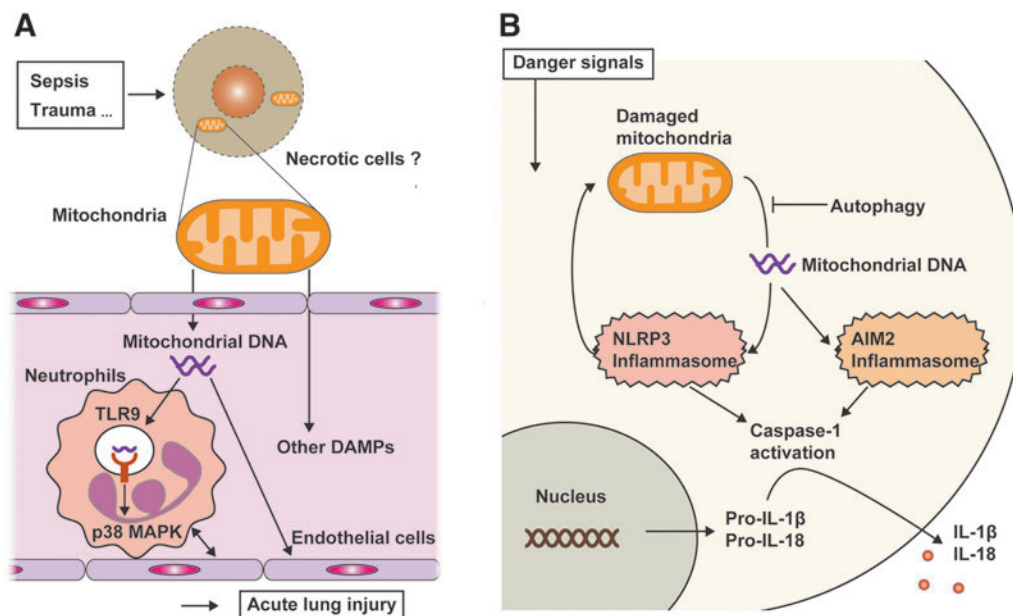


FIG. 3. Released mtDNA activates TLR9 and inflammasome. (A) MtDNA can be released to the cytosol or extracellular space when cells receive various stresses, including mechanical stress or infection. Released mtDNA activates the TLR9-mediated signaling pathway in immune cells such as neutrophils and initiates the proinflammatory cascade to produce proinflammatory cytokines. Extracellular mtDNA also causes adherence of neutrophils and endothelial cells by activating their adherence molecules (*e.g.*, ICAM1 or e-selectin in endothelial cells; CD18 or L-selectin in neutrophils). These events result in systemic endothelia permeability. (B) In the normal condition, aged or damaged mitochondria in cells are eliminated by the autophagy machinery, called mitophagy, to avoid excess generation of oxidative stress. However, when autophagy (mitophagy) is inhibited or the number of damaged mitochondria is beyond the capacity of the autophagy machinery, mtDNA is released into the cytosol. The cytosolic mtDNA activates NLRP3 inflammasome-mediated caspase-1 activation, leading to the secretion of IL-1 β and IL-18. Cytosolic mtDNA can also activate AIM2-dependent inflammasome. AIM2, absent in melanoma 2; ICAM1, intercellular adhesion molecule 1; IL-1 β , interleukin-1 beta; mtDNA, mitochondrial DNA. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

member of this family and is activated by a wide range of signals of pathogenic and nonpathogenic origins (30). Importantly, NLRP3 is also activated by host cell-derived molecules, including extracellular adenosine triphosphate (ATP), hyaluronan, amyloid- β fibrils, and uric acid crystals (30). In addition, the NLRP3 inflammasome is activated by cytosolic mtDNA released from damaged mitochondria in host cells (Fig. 3B) (107). Mitochondria of the cell treated with lipopolysaccharide (LPS) and ATP display severe swelling and destruction of the cristae structure (107). Extracellular ATP treatment rapidly induces mitochondrial dysfunction, including mitochondrial membrane potential transition and generation of mitochondrial ROS in LPS-primed macrophages by activating P2X7 receptor (106, 107). In normal conditions, autophagy, an intracellular process to maintain cellular homeostasis by facilitating the turnover of damaged organelles (104, 126), can remove the dysfunctional mitochondria (mitophagy) (6, 170). However, when the autophagy machinery is inhibited, the dysfunctional mitochondria are accumulated with increased generation of mitochondrial ROS, which leads to mtDNA release into the cytosol and further caspase-1 activation (52, 107) (Fig. 3B). Importantly, exogenous treatment of mtDNA enhances secretion of interleukin-1 beta (IL-1 β) in macrophages treated with LPS and ATP, while delivery of DNase I into the cytosol inhibits cytokine secretion (107). In contrast, nuclear DNA is not translocated to the cytosol during this response (107). Similar to the results observed in DNase II-deficient mice with heart failure (111), the accumulation of mtDNA due to the failure of proper elimination of mitochondria can promote immune responses. In addition, autophagy- or DNase-dependent mtDNA digestion may be an important cellular mechanism in the prevention of mitochondrial DAMP-mediated immune responses. In fact, both mice lacking autophagy protein (*e.g.*, LC3B) or DNase II display increased proinflammatory responses and are more susceptible to septic shock or cardiac failure (74, 107, 111).

Recent studies suggest that cytosolic mtDNA promotes cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING)-mediated type I interferon (IFN) production (131, 163, 164). Moderate mtDNA stress elicited by TFAM deficiency engages cytosolic antiviral signaling to enhance the expression of a subset of IFN-stimulated genes (163). Mechanistically, aberrant mtDNA packaging promotes escape of mtDNA into the cytosol, where it engages the DNA sensor cGAS and promotes STING-IRF3-dependent signaling to potentiate type I IFN responses (163). Interestingly, the mtDNA-released mitochondria also activate the proapoptotic caspase pathway simultaneously, leading to inhibition of type I IFN production (131, 164). Thus, mitochondria play a central role to determine whether dying cells trigger mtDNA-mediated inflammatory responses or remain immunologically silent responses through apoptotic caspases (131, 163, 164).

The translocation of mtDNA from mitochondria to the cytosol was reported in Patrushev *et al.* (118). The mechanism of mtDNA release includes induction of mitochondrial permeability transition pore as cyclosporine A, a potent inhibitor of pore opening, inhibits translocation of mtDNA from mitochondria to the cytosol (70, 107, 118, 119). The release of nucleic acids into the extracellular space has been thought to be related to the cell death, including apoptosis and necrosis (139). Although the concise mechanisms by which types of cells secrete mtDNA remain unclear, secretion of mtDNA to

blood is increased in animal models involved with necrosis or necroptosis (*e.g.*, cecal ligation and puncture, hemorrhagic shock, and irradiation) (38, 41, 178). In addition to the involvement of cell death, mtDNA is also released to the extracellular space in response to microbial killing stimuli, such as LPS + granulocyte-macrophage colony-stimulating factor (75), immunoglobulin E (IgE) + anti-IgE, and LPS + IL-5, respectively, from immune cells such as eosinophils or neutrophils (171, 172, 176). MtDNA is also secreted from activated platelets, leading to promote leukocyte activation (10). MtDNA administered into the peritoneal space of rats is able to be detected in the serum, suggesting that mtDNA released locally can reach the systemic circulation (176). Although concise kinetics of mtDNA *in vivo* remain unclear, these data also suggest the possibility that circulating mtDNA may originate from a wide range of cell types or tissues.

Finally, it should be noted that mtDNA may have unique biological roles compared with nuclear DNA. For example, while the levels of circulating mtDNA dramatically increased after trauma/hemorrhagic shock in rats, the increase of circulating nuclear DNA is modest (178). Functionally, p38-mediated secretion of matrix metalloproteinase (MMP)-8 and -9 is triggered by mtDNA treatment, but not by nuclear DNA (178). Furthermore, injection of mtDNA causes lung injuries or arthritis; however, nuclear DNA has no effect on the development of these tissue injuries *in vivo* (25, 177). The concise mechanisms by which mtDNA and nuclear DNA exert these differential immune responses remain unclear. Further studies will be needed to better understand these mechanisms.

Adenosine triphosphate

ATP is the primary source of energy in all living cells by donating a phosphate group during biochemical activities. ATP is synthesized mainly in mitochondria through activation of the glycolysis pathway and tricarboxylic acid (TCA) cycle. ATP is often considered as the molecular unit of currency of intracellular energy transfer. ATP was first thought to be an intracellular energy source, however, later proved to be an important extracellular signaling molecule. ATP is activated by the activation of purinergic receptor subtype, including P2X receptor (P2XR) or P2Y receptor (P2YR) (144, 154). The genetic variance of P2XR or P2YR is associated with various human diseases (47, 146). Recent studies demonstrate that the increase of extracellular ATP levels also critically contributes to pathogenesis of diseases through the activation of the P2XR or P2YR (144, 154). In normal states, secretion of ATP and its extracellular concentration are tightly regulated by ubiquitous ecto-ATP/ADPases (CD39) (91). ATP can be released from various types of cells, including epithelial, endothelial, red blood cells, and immune cells (11, 150). For example, ATP is secreted from damaged epithelial cells induced by mechanical stimuli (55, 169), hypotonic condition (60), or bacterial infection (134). The functions of extracellular ATP include muscle contraction and relaxation, vasodilatation, neurotransmission, platelet aggregation, ion transport regulation, cell growth, and immune response (16, 32, 151).

Of note, the roles of extracellular ATP in immunity and inflammation have been extensively studied in various immune cells, including macrophages, DCs, neutrophils, and

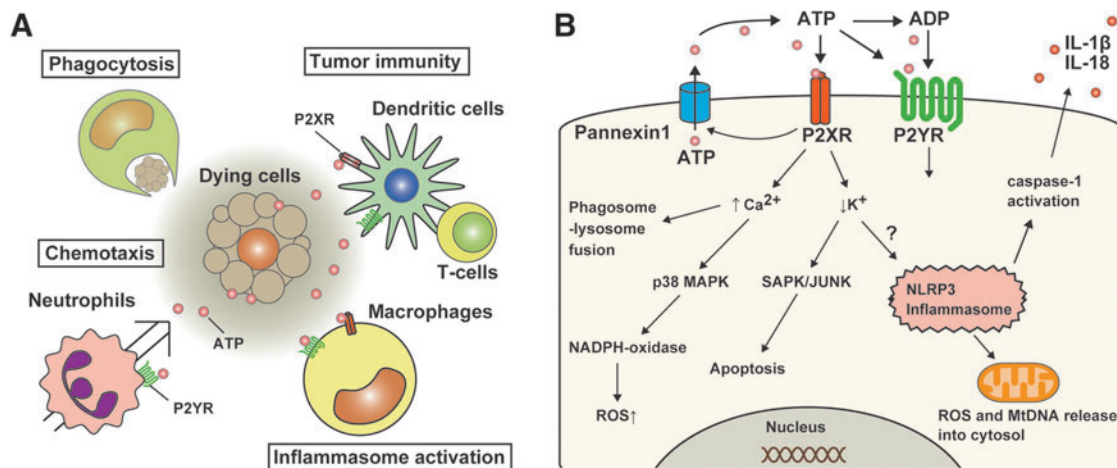


FIG. 4. Critical roles of extracellular ATP on immune responses. (A) Extracellular ATP has diverse effects on various immune cells, such as macrophages, neutrophils, or T cells. The functional roles of ATP are mediated by P2XR or P2YR. ATP activates NLRP3 inflammasome and promotes secretion of IL-1 β and IL-18 in macrophages primed with TLR ligands. ATP also promotes migration and transmigration of immune cells to inflamed sites, killing bacteria, and chemotaxis. The roles of ATP on DCs include maturation and migration of DC and cytokine production. (B) ATP-mediated P2XR or P2YR activation leads to various cellular events. Upon activation of P2XR and P2YR by ATP, potassium efflux and calcium mobilization rapidly occur, which induce activation of NLRP3 inflammasome or MAPKs, leading to initiation of proinflammatory cascade. ATP treatment also causes NADPH oxidase-dependent ROS generation and promotes fusion of phagosome and lysosome, resulting in promoting bacterial killing. DC, dendritic cell; MAPK, mitogen-activated protein kinase; NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

lymphocytes (11, 102) (Fig. 4A, B). For example, in neutrophils, the presence of extracellular ATP triggers neutrophil chemotaxis and release of CXCL8 (also known as IL-8) and elastase. ATP also promotes adhesion of neutrophils to endothelial cells by upregulating the β 2-integrin macrophage antigen (Mac)-1 (CD11b/CD18) (11). Against invading microbes, ATP promotes phagocytosis of pathogens by upregulating Mac-1 (103, 175) and promotes the degranulation of granules (99, 100) and increases generation of ROS (61, 105, 107). In addition, neutrophils release ATP during their inflammatory activation and may enhance those immune responses in an autocrine or paracrine manner. In monocytes, ATP promotes not only adhesion but also subsequently promotes transmigration through the endothelium by shedding of L-selectin from the surface of monocytes (84, 145). ATP also promotes monocyte migration through CD39 (50). One of most studied roles of ATP in macrophages and monocytes is the secretion of IL-1 β and IL-18 through NLRP3 inflammasome activation (Fig. 4B). Binding of ATP to P2X7R causes opening of the P2X7 channel and triggers rapid potassium efflux, resulting in cleavage of caspase-1 (30, 125). Activation of caspase-1 catalyzes the formation of mature IL-1 β and IL-18 from pro IL-1 β and pro IL-18 (30, 125). Furthermore, ATP treatment also generates prostaglandin E2 (83) and promotes fusion of phagosome and lysosome to promote bacterial killing in macrophages (42) (Fig. 4B).

In addition to the roles of ATP in innate immune, ATP is involved in adaptive immune responses. Extracellular ATP induces shedding of L-selectin from T lymphocytes by activation of P2X7 receptor (67), suggesting the role of ATP in migration of activated lymphocytes to sites of inflammation (11). ATP is also involved in proliferation of lymphocytes and negatively regulates effector function of CD4⁺ Th cells (11). Similar to other immune cells, T lymphocytes release

ATP upon activation (93). In B cells, low concentration of ATP (10–100 μ M) triggers B-cell activation, presumably through increased concentration of cytosolic-free Ca²⁺ (114). Thus, extracellular ATP has various immunological roles depending on its extracellular concentration and targeting of immune cells.

The roles of extracellular ATP have been studied in various disease models, such as cancer, asthma, idiopathic pulmonary fibrosis (IPF), or graft-versus-host disease (GVHD) (Table 1). ATP is secreted from dying tumor cells through the autophagic machinery and promotes the recruitment of immune cells, which improves the efficacy of antineoplastic chemotherapy (95, 101). Increased levels of extracellular ATP are observed in bronchial alveolar lavage fluid (BALF) of asthmatic humans and mice sensitized with ovalbumin and alum (65). Extracellular ATP triggers and maintains asthmatic airway inflammation by activating DCs in the mice (65). T helper 2 (Th2) cytokine production and bronchial hyper-reactivity are suppressed when ATP is neutralized by apyrase or cotreated with broad-spectrum P2-receptor antagonists (*i.e.*, suramin) (65). Similarly, exposure of human bronchial cells to allergen extracts from the fungus *Alternaria alternata* induces extracellular ATP release, increased concentration of intracellular Ca²⁺, and secretion of IL-33 (79). The released IL-33 further triggers Th2 cell responses *in vitro* and *in vivo* after the exposure with *A. alternata* (79). The secretion of IL-33 is mediated by autocrine ATP-mediated activation of P2X7R, which is inhibited by antagonists for P2X7R such as oxidized ATP or the gene knockdown of P2X7R (79). Increased levels of extracellular ATP are also observed in the peritoneal fluid after total body irradiation and observed during the development of GVHD in mice and in humans (165). Stimulation of antigen-presenting cells with ATP enhances proinflammatory responses such as IFN- γ production

and donor T-cell expansion (165). Neutralization of ATP by apyrase or genetic deficiency of P2X7R during irradiation-induced GVHD development improved survival of the mice (165). The levels of ATP in BALF are also elevated in mice treated with bleomycin, animal models of lung inflammation and fibrosis (129). Intranasal injection of ATP further enhances the bleomycin-induced lung inflammation, while administration of suramin and apyrase inhibits the inflammation of lungs in the mice (129). P2X7R knockout mice display attenuated lung inflammation and fibrosis after bleomycin treatment (129). Since extracellular ATP activates NLRP3 inflammasome through P2X7R, the pathogenesis of bleomycin-induced fibrosis may include the pathway of ATP-P2X7R-NLRP3 inflammasome. Thus, these reports consistently show that inhibition of ATP-mediated P2XR or P2YR signaling pathways ameliorates pathogenesis of the disease, including asthma, fibrosis, or GVHD, suggesting that extracellular ATP is a critical mitochondrial DAMP to promote pathogenesis of various diseases.

Mitochondrial transcription factor A

Mitochondrial transcription factor A (mtTFA, mtTF1, TFAM), a member of the HMG box family, is an essential protein that binds mtDNA in a sequence-independent manner to regulate both mitochondrial transcription initiation and mtDNA copy number (17, 72). The activation of mitochondrial promoters is regulated by the protein levels of TFAM (17, 72). TFAM also binds mitochondrial genome nonspecifically to ensure proper maintenance of mtDNA (17, 72). Heterozygous knockout of TFAM in mice shows reduced mtDNA copy number and respiratory chain deficiency in the heart (86). Furthermore, homozygous knockout of TFAM in mice displays severe mtDNA depletion and impairment of oxidative phosphorylation, resulting in an early embryonic lethality (86). Mice with adipose-specific deletion of TFAM exhibit the higher energy expenditure and are protected from age- and diet-induced obesity, insulin resistance, and hepatosteatosis, despite a greater food intake (162). TFAM in RKO rectal cancer cell lines carrying a TFAM-truncating mutation suppresses cell proliferation and inhibits RKO cell-induced xenograft tumor growth in nude mice (56). Thus, TFAM plays critical roles in mitochondrial functions and pathophysiological condition by regulating a copy number of mtDNA. Similar to HMGB1, an important member of DAMPs (21), TFAM is also involved in immune responses. While a recent study shows that intracellular TFAM negatively regulates antiviral immune response (163), liberated or extracellular TFAM has been shown to promote inflammatory responses. For example, costimulation with NFPs and TFAM synergistically enhances the cytokine secretion (27). Similarly, the effect of CpG DNA on tumor necrosis factor (TNF) secretion in splenocytes is augmented by additional treatment of TFAM (68). These observations suggest that extracellular TFAM in the presence of other mitochondrial DAMPs can synergistically enhance immune responses. *In vivo*, the serum level of TFAM is elevated in rats subjected to hemorrhagic shock, a model to induce cell death, including necrosis (20). Importantly, administration of recombinant TFAM increases the levels of IL-6 and TNF both in serum of rats and in the media of RAW264.7 macrophages (20). Furthermore, intravenous treatment of TFAM increased myeloperoxidase activity

in lung and serum levels of lactate in the rats (20), a currently used biomarker for critically ill patients (1). Thus, these reports suggest that extracellular TFAM can promote inflammatory responses as DAMPs.

N-formyl peptide

Several natural formylated peptides purified from bacteria have been identified as low-molecular-weight chemoattractants to activate human phagocytes (19, 87).

Formylated peptides are recognized by formyl peptide receptors (FPRs), members of the seven transmembrane G protein-coupled receptors superfamily and highly expressing on polymorphonuclear and mononuclear phagocytes (87, 115). In particular, the NFP has been extensively studied due to its diversity of immune functions, such as phagocytosis, generation of reactive oxygen intermediate, and release of proteolytic enzymes (115). Unlike other leukocyte chemoattractants, NFP originates from either mitochondrial proteins of ruptured or dying host cells or microbes such as *Escherichia coli* (87). This unique character of NFPs suggests that the NFP is involved in host defense against bacterial infection and in the clearance of damaged cells. The immunomodulatory effect of NFPs was first reported as a chemoattractant for neutrophils (19) and subsequently observed in platelets (28). Treatment with purified NFPs isolated from mitochondria promotes chemotaxis of human PMN, whereas nonformylated mitochondrial proteins have no effect (19). Similarly, the synthetic peptides derived from human mitochondrial proteins or *Listeria monocytogenes* induce chemotaxis (124). Furthermore, knockout of formyl peptide receptor 1 (FPR1), a representative receptor for NFPs, increases susceptibility with *L. monocytogenes* in mice (48), suggesting that NFP is important for antibacterial immune responses. The roles of NFP on inflammation have been also reported. For example, combined treatment of antagonists for FPR1 and CXC chemokine receptor 2 improved acetaminophen-induced inflammatory responses and liver injury in mice (97). The mechanism by which NFP induces chemotaxis is mediated by increase of Ca²⁺ influx through FPRs (87). The functions of activated FPRs upon ligation of formylated peptides include morphological polarization, locomotion, production of ROS, and release of proteolytic enzymes (115). In humans, dysfunctional variant FPR alleles are associated with localized juvenile periodontitis (57). Neutrophils from the patients with LJP display a reduced chemotaxis in response to NFPs (29, 161), suggesting important immunological roles of NFPs in humans. The NFP also promotes chemotaxis by secreting IL-8, a potent chemoattractant (27). Costimulation with NFP and mtDNA synergistically increases IL-8 secretion (179). Importantly, the NFP is selectively secreted from necrotic cells (*e.g.*, hypoxia-mediated necrosis), but not from apoptotic cells (*e.g.*, staurosporine-mediated apoptosis), and triggers chemotaxis (28). These data suggest that the necrotic cells are the critical sources of extracellular NFPs.

Succinate

Succinate, an intermediate synthesized in the TCA cycle, donates electrons to the electron transport chain and therefore is essential for maintaining mitochondrial functions. It has been shown that succinate synthesized in mitochondria can be secreted to the extracellular space *in vitro* (141). The levels of succinate in cell culture media are increased by

incubation with antimycin, an inhibitor for mitochondrial electron transport specifically between cytochromes, b and c1, and an inducer of cell death, including necrosis (141). Interestingly, rotenone, another potent inhibitor for the transfer of electrons from iron–sulfur centers in complex I to ubiquinone, has no effect on the secretion of succinate (141). Importantly, extracellular succinate can act as a signaling molecule in both innate and adaptive immune systems. Succinate treatment triggers an intracellular calcium mobilization and a migratory response and also synergistically enhances the production of proinflammatory cytokines (*e.g.*, TNF and $\text{INF-}\gamma$) induced by TLR ligands in DCs (132). Succinate also enhances antigen-specific activation of human and mouse helper T cells through the G protein-coupled receptor GPR91 of DCs (132) (Fig. 5A). GPR91-deficient allografts elicit a weaker transplant rejection than did the corresponding grafts from wild-type mice (132). Succinate also promotes innate immune response, particularly increases $\text{IL-1}\beta$ gene expression (157) (Fig. 5B). LPS increases the production of succinate by activating glycolysis activity, which contributes to $\text{IL-1}\beta$ production in macrophages by stabilizing hypoxia-inducible factor 1-alpha ($\text{HIF-1}\alpha$) (157). Exogenous treatment of succinate enhances $\text{IL-1}\beta$ production, but not IL-6 and TNF in LPS-primed macrophages, while $\text{HIF-1}\alpha$ deficiency impairs the effect of succinate on $\text{IL-1}\beta$ production (157). Succinate is also involved in pathogenesis of pulmonary artery hypertension (PAH) (46, 168). The expression of GPR91 and phosphorylated Akt (p-Akt) in the right ventricle significantly increases in a rat model of pulmonary artery banding PAH model (168). Administration of succinate further increases the p-Akt levels and aggravates

right ventricular hypertrophy *in vivo* (168), although the roles of the succinate-mediated GPR91 signaling pathway on immune responses in the PAH model remain unclear. Thus, these reports suggest that extracellular succinate can play critical roles in pathogenesis of various disease models.

Cardiolipin

Cardiolipin is a lipid dimer consisting of two phosphatidyl groups bridged by a glycerol in mitochondria and is important for a diverse range of mitochondrial functions (24). Cardiolipin is required for maintaining of the optimal functions of a number of mitochondrial proteins and processes such as mitochondrial respiration and mitochondrial biogenesis (23). Therefore, it is possible that deregulation of cardiolipin metabolism contributes to various pathological conditions (23, 117). The abnormality of cardiolipin metabolism is associated with human diseases such as ischemic conditions, hypothyroidism, aging, and heart failure (23, 117). During death receptor-mediated apoptosis, cardiolipin moves from mitochondria to the cell surface and the other intracellular organelles (147, 148). Cardiolipin regulates cytochrome c release and mitochondrial outer membrane permeabilization (113), suggesting the role of cardiolipin in cell death. In addition, cardiolipin confers immunological effects. Cardiolipin binds to CD1d and stimulates CD1d-restricted $\gamma\delta$ T cells (35). Cardiolipin is also required for NLRP3 inflammasome activation (66). Cardiolipin binds to NLRP3 directly and activates inflammasome-mediated immune responses (66). Similar to NFPs, cardiolipin is enriched in both mitochondria and bacterial membranes (78, 81, 90). Therefore, it is

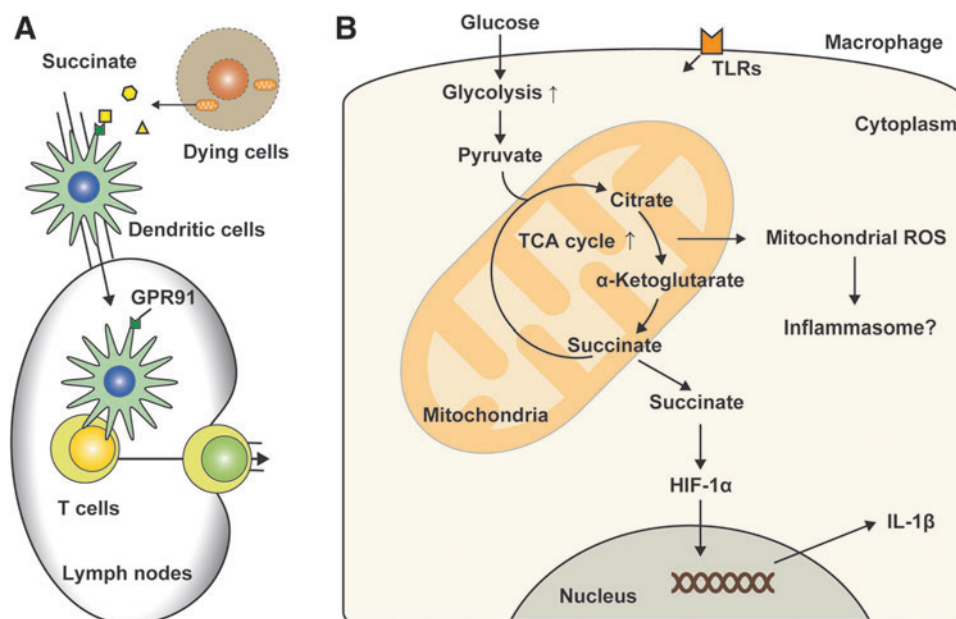


FIG. 5. Mitochondrial metabolite in immune cells. (A) Succinate is an intermediate produced during TCA cycle in mitochondria and can secrete to the extracellular space when mitochondrial function is disturbed. Extracellular succinate enhances antigen-specific activation of helper T cells through GPR91 of DCs. (B) Glycolysis is a metabolic pathway that provides intermediates for energy generation. The induction of glycolysis by TLR ligands promotes $\text{IL-1}\beta$ gene expression by succinate-mediated stabilization of $\text{HIF-1}\alpha$ in macrophages. In addition, high concentrations of glucose increase $\text{IL-1}\beta$ secretion in an NLRP3-dependent mechanism. However, the concise mechanism by which activation of glycolysis-TCA cycle contributes in activation of inflammasome is still unclear. $\text{HIF-1}\alpha$, hypoxia-inducible factor 1-alpha; TCA, tricarboxylic acid. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

speculated that cardiolipin can act as PAMPs and DAMPs. In normal condition, cardiolipin comprises only ~1–2% of alveolar surfactant and its levels are elevated in acute lung injury models (81, 90). Importantly, exogenous treatment of cardiolipin impairs the surface tension-lowering activity in mice (127). The individuals with progressive familial intrahepatic cholestasis type 1 (PFIC1 or Byler's disease) have mutations in ATP8b1 and the increased risk for pneumonia (127). Atb8b1 binds extracellular cardiolipin and imports into intracellular spaces to remove cardiolipin from the extracellular space. Gene transfer of *atp8b1* or treatment of the peptide, including ATP8b1, improves acute lung injuries induced by *E. coli* in mice (127). In humans, the levels of cardiolipin in tracheal aspirates are elevated in patients with pneumonia (127). Although it is still unclear whether the increased cardiolipin in patients is derived from host mitochondria or bacteria, these results suggest that extracellular cardiolipin is a critical factor in the pathogenesis of acute lung injuries.

The Mitochondrial DAMPs in Human Diseases

The levels of mtDNA in human diseases

While the roles of mitochondrial DAMPs are studied in a wide range of experimental models, the association between mitochondrial DAMPs and human diseases also has been reported. Table 2 demonstrates the list of mitochondrial DAMPs in which levels are changed in the patients with various diseases.

The levels of circulating mtDNA are increased in patients with diseases, such as sepsis (82, 167), strokes (160), acute liver failure (97), massive pulmonary embolism (4), or cancers (39, 98, 174), and are also associated with severities or prognosis of the diseases (Table 2). While the levels of circulating mtDNA are elevated in the patients with urological cancers (39, 98) and ovarian cancer (174), the levels of mtDNA are not increased in patients with Ewing sarcoma (173) or breast cancer (77). It is likely that the changes of circulating mtDNA may be dependent on types of cancers or sarcomas. ATP is also elevated in plasma, serum, or BALF from patients with various diseases (Table 2). Importantly, there are many cases where the association between the levels of mitochondrial DAMPs and the severities of human diseases is consistent or similar to that observed *in vivo* studies. For example, while the elevation of ATP in BALF is observed in patients with IPF (129) or asthma (65), the elevated levels of ATP are also observed in BALF from the experimental mice models of fibrosis (129) or bronchial hypersensitivity (65). Similarly, the levels of circulating cell-free mtDNA are elevated in patients with trauma (167, 179) or sepsis (82, 167) and also observed in animal models of trauma/hemorrhagic shock (178) or bacterial sepsis (155). In addition, the exogenous administration of mitochondrial DAMPs is able to develop the similar pathological conditions with human diseases in animals. Intratracheal injection of ATP abrogates the pathogenesis of bleomycin-induced fibrosis in mice, whereas the levels of ATP in BALF are further elevated in patients with exacerbated IPF compared with stable IPF (129). The levels of mtDNA are elevated in both plasma and synovial fluid of patients with rheumatoid arthritis (58), and the administration of mtDNA develops arthritis in mice (25). These data suggest that mitochondrial DAMPs elevated in the patients may be involved in the pathogenesis of human diseases.

MtDNA: a biomarker in medical intensive care unit

Circulating mtDNA levels are higher in patients admitted in medical intensive care units (ICUs), who died within 28 days of the medical ICU admission, and are also higher in patients with diagnoses commonly associated with mortality in the ICU such as sepsis and acute respiratory distress syndrome (ARDS) (108). Medical ICU patients with elevated mtDNA levels have an increase in their odds of dying within 28 days of ICU admission, although there is no evidence that the association between the mtDNA level and medical ICU mortality is attenuated in models adjusted for clinical covariates, including acute physiology and chronic health evaluation (APACHE) II score, sepsis, or ARDS (108). Furthermore, mtDNA can improve risk prediction even after accounting for the levels of lactate or procalcitonin and APACHE II score among the medical ICU patients (108). Similar findings are observed in the patients presenting at the emergency room. The levels of mtDNA in plasma are elevated in patients with sepsis and only the plasma mtDNA level is independently predictive of fatality among the variables used in the logistic regression (*e.g.*, mechanical ventilation, mean sequential organ failure assessment score, serum lactate, and plasma mtDNA) (82). Importantly, in contrast to the strong association between mtDNA and the mortality in the patients admitted in medical ICUs, no evidence of association is observed in patients in nonmedical ICU patients such as surgical ICU, although there is no evidence that the mtDNA level is higher in patients from medical ICUs when compared with surgical ICUs (108). MtDNA could be released from cells or tissue as the result of surgery, and in this setting, an elevated mtDNA level may be unlikely to be associated with an increased mortality.

Since mtDNA acts as a potent activator of inflammasome (107), it is possible that the inflammasome is also associated with pathogenesis of critical care illness. In fact, the levels of circulating IL-18, a representative inflammasome-mediated proinflammatory cytokine, are elevated among the patients with sepsis and ARDS and are associated with the disease severity and mortality (37, 53, 110). Importantly, the plasma levels of mtDNA and IL-18 are positively associated among the medical ICU patients (108).

The levels of mtDNA are decreased in mononuclear cells from septic patients compared with healthy volunteers, and the cellular mtDNA copy numbers are negatively associated with APACHE II score (122), suggesting the possible sources of circulating cell-free mtDNA in critically ill patients. In addition, a recent study suggests that platelets can secrete mitochondria upon platelet activation (10), which can also explain the increased levels of extracellular mtDNA reported in blood in various pathological conditions. However, at this point, it remains unclear which type of cells are responsible for secretion of mtDNA into blood in those critically ill patients. Further studies will be needed to clarify this point. In summary, circulating cell-free mtDNA could be a valuable addition to assessment of patients in the ICU or emergency room and point the way to the possibility of new diagnostic and/or therapeutic approaches for patients with critical illness.

ATP: a biomarker in lung diseases

While the roles of extracellular ATP and P2X7R have been reported in various experimental disease models, clinical relevance of ATP and P2XR or P2YR is also studied in patients.

TABLE 2. ASSOCIATION WITH HUMAN DISEASES

<i>Mitochondrial DAMPs</i>	<i>Diseases</i>	<i>Specimen</i>	<i>The levels of DAMPs in diseases. Association with clinical phenotypes</i>	<i>References</i>
mtDNA	Rheumatoid arthritis	Plasma Synovial fluid	The levels of mtDNA are higher in the patients with RA than in the control subjects.	(58)
	Urological malignancies	Serum	The levels of mtDNA are higher in the patients with urological malignancies (bladder cancer, renal cell carcinoma, or prostate cancer) than in the healthy subjects.	(39)
	Prostate cancer	Plasma	The levels of mtDNA are higher in the patients with prostate cancer than the patients with benign diseases. The levels of mtDNA are correlated with hemoglobin count and the level of PSA, a tumor marker for prostate cancer.	(98)
	Ovarian cancer	Plasma	The cancer patients who have higher levels of mtDNA decrease the survival compared with the cancer patients with lower levels of mtDNA.	(174)
	Breast cancer	Plasma	The levels of mtDNA are elevated in patients with epithelial ovarian cancer, but not with benign epithelial ovarian tumor, compared with the healthy control group.	(77)
	Ewing sarcoma	Serum	The levels of mtDNA are lower in the patients with breast cancer than control groups. (The levels of nuclear DNA are higher in the patients.)	(173)
	Myocardial infarction (MI)	Plasma	The levels of mtDNA are lower in the patients with Ewing sarcoma than in the healthy subjects	(9)
	Exposure to haloalkanes	Serum	The levels of mtDNA are higher and sustained in patients with MI than with stable angina pectoris. The levels of mtDNA are higher in patients with transmural MI than with nontransmural MI.	(15)
	HIV infection	Plasma	The levels of mtDNA (both 230 and 79 bp) are higher in the group with exposed haloalkane-based pesticides than the control subjects.	(26)
	Patients in ER	Plasma	The levels of mtDNA are higher in the HIV-positive patients than the healthy controls and the long-term nonprogressors.	(82)
	Patients in ER	Plasma	The levels of mtDNA are correlated with plasma viral load.	(121)
	Patients in medical ICU	Plasma	The levels of mtDNA are higher in the patients with severe sepsis on admission to the ER than the control patients.	(108)
	Trauma	Plasma	The levels of mtDNA are higher in the nonsurvivors than in the survivors.	(179)
	Femur fracture reamings	Plasma	The levels of mtDNA are negatively associated with organ dysfunction.	(59)
	ACABM	Plasma	The levels of circulating cell-free mtDNA are associated with disease severity in critically ill patients. The medical ICU patients who have higher levels of plasma mtDNA decrease the survival compared with the patients with lower levels of mtDNA.	(94)
	Acute ischemic stroke	Plasma	The levels of mtDNA are higher in the patients with trauma than the control subjects.	(160)
	Out-of-hospital cardiac arrest	Plasma	The levels of mtDNA are higher in the patients with femur fracture reamings than control volunteers.	(3)
	Acute liver failure	Serum	The levels of mtDNA are higher in the patients with bacterial meningitis than with aseptic meningitis and the normal volunteers.	(97)
	Massive PE	Plasma	The levels of mtDNA are negatively correlated with the modified Barthel index, a scale to measure performance in activities of daily living. Higher levels of mtDNA are associated with poor outcome in patients with ACABM	(4)
	Maintenance hemodialysis	Plasma	The levels of mtDNA are elevated in patients with acute ischemic stroke compared with the control subjects.	(18)
	Aging	Plasma	The levels of mtDNA are elevated in nonsurvivors than in survivors after out-of-hospital cardiac arrest. The levels of mtDNA are elevated in patients with acute liver failure and are associated with liver dysfunction marker ALT levels.	(120)
			Plasma mtDNA concentrations are higher in patients with massive PE than in patients with submassive PE or controls.	
			The levels of mtDNA are elevated in patients with maintenance hemodialysis compared with healthy subjects.	
			MtDNA plasma levels increased gradually after the fifth decade of life and the levels of mtDNA are associated with the levels of proinflammatory cytokines.	

(continued)

TABLE 2 (CONTINUED)

Mitochondrial DAMPs	Diseases	Specimen	The levels of DAMPs in diseases. Association with clinical phenotypes	References
ATP	COPD	BALF	ATP concentration is the highest in the patients with COPD, even after smoking cessation.	(92)
	Asthma	BALF	ATP concentration is negatively associated with lung function and positively with BALF neutrophil counts. ATP concentration is elevated in the patients with asthma who undergo segmental allergen provocation compared with the control patients.	(65)
	Cystic fibrosis	Plasma	ATP concentration is elevated in patients with CF compared with control healthy subjects.	(85)
		Sputum, BALF, EBC	ATP concentration is elevated in patients with CF compared with disease control children in BALF, sputum, and EBC. ATP concentration is inversely related to lung function and strongly correlated with neutrophil counts in BALF.	(40)
	IPF	BALF	ATP concentration is elevated in patients with IPF compared with control patients.	(129)
	Type 1 diabetes	Plasma	ATP concentration is elevated in patients with type 1 diabetes compared with control healthy subjects. ATP concentration was negatively correlated with coronary flow velocity response after intervention.	(76)
		Ascites	ATP concentration is elevated in patients with GVHD compared with patients without GVHD who have HCT or patients who do not have HCT.	(165)
	PAACG	Aqueous humor	ATP concentration is elevated in patients with PAACG associated with intraocular pressure.	(89, 180)
	AMD with subretinal hemorrhage	Vitreous	ATP concentration is associated with intraocular pressure.	(109)
		Red blood cells	ATP release is impaired in red blood cells of patients with primary pulmonary hypertension.	(152)
Succinate	Metabolic acidosis	Plasma	The levels of succinate are elevated in patients with metabolic acidosis, including diabetic ketoacidosis and lactic acidosis, compared with control subjects.	(45)
	Acute coronary syndrome	Plasma	The levels of succinate are elevated in patients with non-ST elevation acute coronary syndrome from day 0 (after the diagnosis) to 6 months compared with the healthy control group.	(159)
		Plasma	The levels of succinate are elevated in individuals with <i>PTEN</i> , <i>SDHB</i> , and <i>SDHD</i> mutation.	(62)
	Exercise in patients with type 1 diabetes	Plasma	The levels of succinate are elevated after exercises, including exercise treadmill testing, bicycle ergometer, and marathon.	(88)
		Serum	The levels of succinate are elevated after an intense exercise at 80% of VO_2 max for 30 min.	(14)
Cardiolipin	Pneumonia	Tracheal aspirates	The levels of cardiolipin are higher in patients with pneumonia than critically ill patients with nonpulmonary illnesses or patients with congestive heart failure.	(127)

ACABM, adult community-acquired bacterial meningitis; AMD, age-related macular degeneration; ALT, alanine transaminase; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; EBC, exhaled breath condensate; ER, emergency room; ICU, intensive care unit; IPF, idiopathic pulmonary fibrosis; HCT, hematopoietic cell transplantation; PAACG, primary acute angle closure glaucoma; PE, pulmonary embolism; PSA, prostate-specific antigen; RA, rheumatoid arthritis; VO_2 max, maximal oxygen consumption.

Of note, recent studies demonstrate the association between the levels of extracellular ATP and the various lung diseases, such as chronic obstructive pulmonary disease (COPD) (92), asthma (65), IPF (129), and cystic fibrosis (40) (Table 2). The levels of ATP in BALF are elevated in patients with COPD compared with smokers or ex-smokers (92). ATP levels in BALF are correlated negatively with lung function and positively with BALF neutrophil counts (92). In addition, blood neutrophils harvested from the patients with COPD show a stronger chemotaxis and an elastase release in response to ATP treatment compared with the control subjects (92). In patients with asthma, in addition to increased levels of ATP in BALF, the functional activity or Single Nucleotide Polymorphism of P2X7R is also associated with the exacerbation or severity of asthma (33, 96). While the pathogenesis of these lung diseases differs from each other, inflammatory responses are commonly involved in the development and acute exacerbation of these diseases. When lung epithelial cells are damaged or injured by

oxidative stress or invading microbes, these cells may secrete ATP into the alveolar space. Subsequently, the released ATP activates P2X7R of immune cells (*e.g.*, alveolar macrophages) and may further enhance inflammatory responses in the lungs. In addition, the activation of P2X7R by ATP often leads to cell death in immune cells (7). Therefore, this cell death may release other DAMPs (*e.g.*, ATP, mtDNA, or NFP) to cytosol or extracellular spaces and further promotes inflammatory response (*e.g.*, inflammasome) in the lung through autocrine or paracrine systems. Finally, these series of inflammatory events may result in the disruption of alveolar structures and functions or abrogation of fibrosis. Thus, it is likely that extracellular ATP is critically involved in inflammatory responses in human lung diseases. Since other mitochondrial DAMPs may also be involved in the lung diseases (138), it would be worth measuring the extracellular levels of other mitochondrial DAMPs in these patients. Measurement of mitochondrial DAMPs, including ATP, may be useful to predict disease severity or outcome.

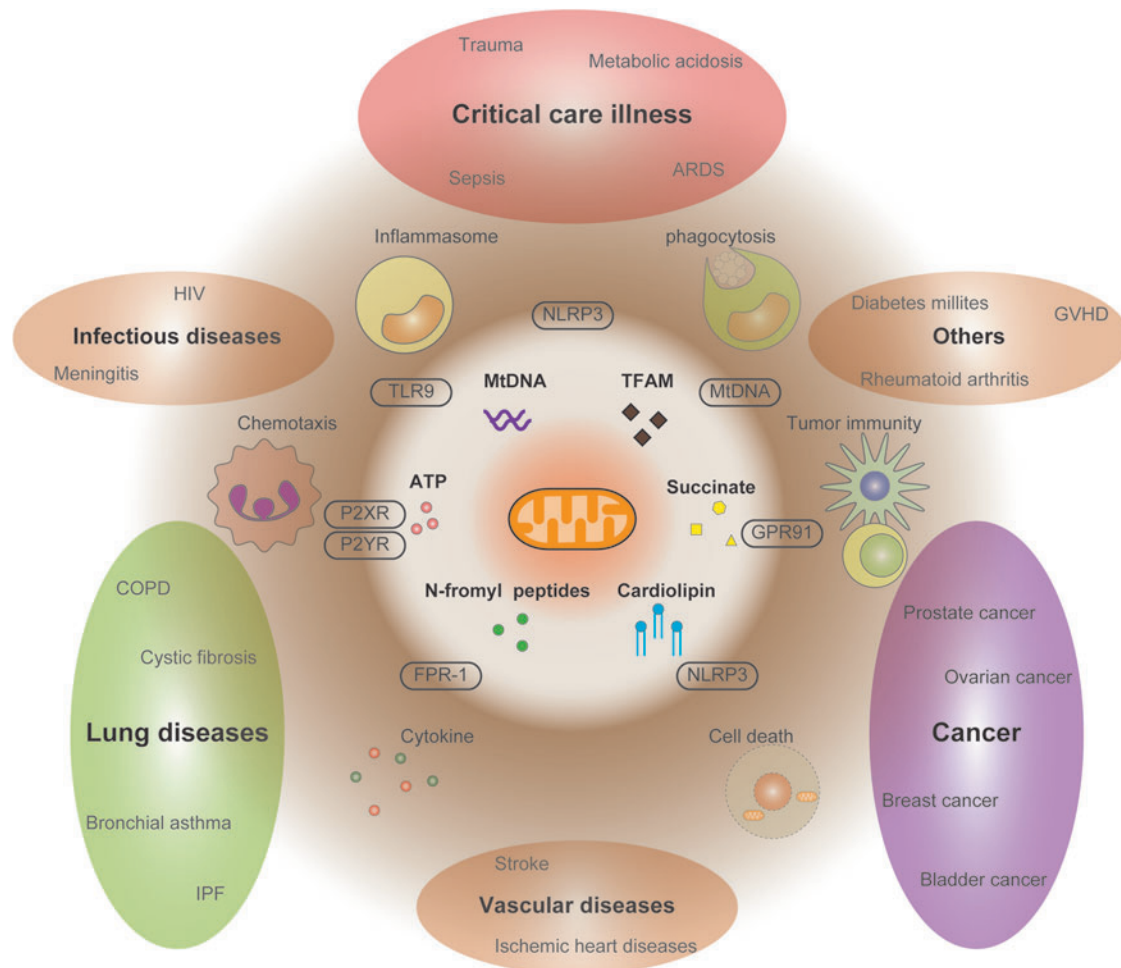


FIG. 6. Diverse effects of mitochondrial DAMPs on disease progression. The functional modes of mitochondrial DAMPs at cellular levels include secretion of proinflammatory cytokines (*e.g.*, inflammasome activation), promoting phagocytosis, triggering chemotaxis, and regulating tumor immunity. Mitochondrial DAMPs are involved in pathogenesis of various diseases. The roles of mitochondrial DAMPs in diseases are shown. Various types of mitochondrial DAMPs may additionally or synergistically contribute to the pathogenesis of the diseases. For example, in sepsis, ATP and NFP promote chemotaxis of neutrophils or monocytes. ATP also activates inflammasome and triggers release of mtDNA into the cytosol, which further enhance caspase-1 activation. In trauma, the secreted mitochondrial DAMPs from damaged tissues, including NFP and mtDNA, activate chemotaxis, secretion of proinflammatory cytokines, and adhesion of neutrophil to the endothelium. NFP, *N*-formyl peptides. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Concluding Remarks

We have reviewed the roles of mitochondrial DAMPs in experimental disease models and also the association between mitochondrial DAMPs and human diseases (Fig. 6 and Table 2). Although there are ample data to support the critical roles of mitochondrial DAMPs in diseases, there are also many questions to be resolved. First, it is unclear how individual mitochondrial DAMPs cross talk with each other and affect immune responses *in vivo* or in patients. For example, *in vitro*, treatment of multiple mitochondrial DAMPs (*e.g.*, TFAM and NFP) synergistically enhances immune function. It is possible that mitochondrial DAMPs may exert potent biological roles when they interact with other mitochondrial DAMPs or other extracellular molecules. Second, it also remains unclear what types of cell death are responsible for release of mitochondrial DAMPs *in vivo* or in patients. Some DAMP molecules such as ATP can be secreted from necrotic cells or apoptotic cells (123, 130). During the development of diseases, the host may have different types of cell death (*e.g.*, apoptosis, necrosis, necroptosis, autophagic cell death, or pyroptosis in various cells or tissues) (7, 34, 44). Third, it is also challenging to elucidate which cell types are responsible for the release of mitochondrial DAMPs and contribute to the immune responses *in vivo*. While a number of reports suggest immune function of extracellular ATP in human lung diseases, it is also important to note that circulating ATP secreted from red blood cells may play a protective role in the pathogenesis of pulmonary hypertension (12, 151, 152). Mitochondrial DAMPs secreted from different types of cells may have different biological roles *in vivo*. Thus, further studies will be needed to elucidate the concise mechanism by which mitochondrial DAMPs contribute to the pathogenesis of human diseases.

Given the critical roles of mitochondrial DAMPs in the pathogenesis of diseases and correlation between the levels of mitochondrial DAMPs and severities of human diseases, it is also worth studying the therapeutic aspect of regulating mitochondrial DAMPs. Regulating the secretion or the signaling pathways of mitochondrial DAMPs may lead to dampening inflammatory response and tissue injuries and further ameliorating the pathogenesis of diseases.

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Date of first submission to ARS Central, June 2, 2015; date of acceptance, June 11, 2015.

Abbreviations Used

ACABM = adult community-acquired bacterial meningitis
 AIM2 = absent in melanoma 2
 ALT = alanine transaminase
 APC = antigen-presenting cells
 APACHE II = acute physiology and chronic health evaluation II

ARDS = acute respiratory distress syndrome
 ATP = adenosine triphosphate
 BALF = bronchial alveolar lavage fluid
 CD4⁺ Th cells = cluster of differentiation 4 (⊕) helper T cells
 CF = cystic fibrosis
 cGAS = cyclic GMP-AMP synthase
 CLP = cecal ligation and puncture
 COPD = chronic obstructive pulmonary disease
 CXCL8 = chemokine (C-X-C motif) ligand 8
 DAMPs = damage-associated molecular patterns
 DC = dendritic cell
 DNase II = deoxyribonuclease II
 EBC = exhaled breath condensate
 ER = emergency room
 FPRs = formyl peptide receptors
 GM-CSF = granulocyte-macrophage colony-stimulating factor
 GPR91 = G protein-coupled receptor
 GVHD = graft-versus-host disease
 HCT = hematopoietic cell transplantation
 HIF-1 α = hypoxia-inducible factor 1-alpha
 HMG = high-mobility group
 ICU = intensive care unit
 IFN- γ = interferon- γ
 IgE = immunoglobulin E
 IL-1 β = interleukin-1 beta
 IPF = idiopathic pulmonary fibrosis
 LOX-1 = lectin-like, oxidized low-density lipoprotein receptor-1
 LPS = lipopolysaccharide
 MAPK = mitogen-activated protein kinase
 MMP = matrix metalloproteinase
 MPO = myeloperoxidase
 mtDNA = mitochondrial DNA
 MTX = mitoxantrone
 NADPH = nicotinamide adenine dinucleotide phosphate
 NF- κ B = nuclear factor kappa-light-chain-enhancer of activated B cells
 NFP = N-formyl peptides
 NLR = NOD-like receptors
 NLRP3 = NLR family, pyrin domain-containing 3
 NOD = nucleotide-binding and oligomerization domain
 OVA = ovalbumin
 P2X7R = P2X purinoceptor 7
 PAACG = primary acute angle closure glaucoma
 PAH = pulmonary artery hypertension
 PAMPs = pathogen-associated molecular patterns
 PE = pulmonary embolism
 PMA = phorbol 12-myristate 13-acetate
 PMN = polymorphonuclear leukocyte
 PSA = prostate-specific antigen
 RA = rheumatoid arthritis
 ROS = reactive oxygen species
 STING = stimulator of interferon genes
 TCA = tricarboxylic acid
 TFAM = mitochondrial transcription factor A
 Th2 = T helper 2
 TLRs = toll-like receptors
 TNF = tumor necrosis factor