The Role of Danger Signals in the Pathogenesis and Perpetuation of Critical Illness

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The Stranger and Danger Models

In 1989, Janeway (1) introduced a conceptual framework to understand how the innate immune system selectively responds to potentially threatening infections. Based on empirical observations, he proposed that the innate immune system must not only distinguish foreign cells from native cells, but also requires the presence of pathogenic costimulatory molecules to initiate inflammatory signaling cascades (1, 2). These signals, typified by gram-negative LPS and termed "pathogen-associated molecular patterns" (PAMPs), are molecularly distinct, foreign particles that serve as necessary immune adjuvants, causing local inflammation and tissue destruction (3). This theory was corroborated by the discovery of a new class of receptors known as PRRs (pattern-recognition receptors) (4, 5). This broad class of receptors, typified by the TLR (Toll-like receptor) family, bind structurally conserved moieties, such as microbial cell wall fragments and foreign DNA (Figure 1) (6). The activation of these receptors is responsible for local inflammation, most distinctly through the induction of multiple pathways, including the TNF (tumor necrosis factor)-α/NF-κB (nuclear factor-κB) signaling cascade as well as NLRP3 (nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3) inflammasome activation with production of inflammatory molecules, such as IL-1β and IL-18 (Figure 2). PRR activation also recruits and activates circulating leukocytes, such as macrophages and neutrophils, which enhance microbial killing through the release of reactive oxygen species, proteases, and IFNγ (7).

This framework, however, fails to explain the systemic inflammatory response observed during noninfectious critical illness, such as major trauma, burns, or cardiac arrest; nor does it explain the prolonged organ dysfunction in sepsis despite clearance of the original infection. In 1994, a complimentary model was proposed, coined the "danger" model (8). Based purely on theoretical grounds, the "danger" model hypothesized that unregulated, necrotic cell death must release endogenous molecules that trigger the innate immune system, leading to local "sterile" inflammation and tissue destruction. Definitive empirical evidence for this concept developed throughout the 1990s by observation of local cellular reaction to necrotic and apoptotic cell death. Several molecules were discovered in this period that can bind to PRRs and initiate local inflammatory responses (9–12). These activators of the innate immune system have been named alarmins, cell death-associated molecules, and damage-associated molecular patterns (DAMPs).

DAMP release has been implicated in the development of a variety of both acute and chronic inflammatory conditions. In critically ill patients, DAMPs and activation of PRR-related pathways are involved in the pathogenesis of the sterile systemic inflammatory response syndrome and perpetuation of the multiorgan dysfunction syndrome (MODS) during sepsis (13, 14) and trauma (15). DAMPs have also been shown to be integral to the development of ventilator-induced acute lung injury (16) and drug-induced liver injury (17), and have been linked to outcomes after cardiac arrest (18).

To separate DAMPs from simple biomarkers or inflammatory cytokines, criteria have been proposed to formally characterize potential DAMPs (19, 20): *1*) rapidly released in response to infection or tissue injury; *2*) have effects on antigen-presenting cells that modulate immune activity; *3*) active as a purified molecule at concentrations in pathophysiological situations; *4*) selective elimination or inactivation should inhibit biological activity; and *5*) have a separate biological role in noninflammatory states. Since the proposal of the initial "danger" hypothesis, numerous host-derived intracellular and extracellular molecules have

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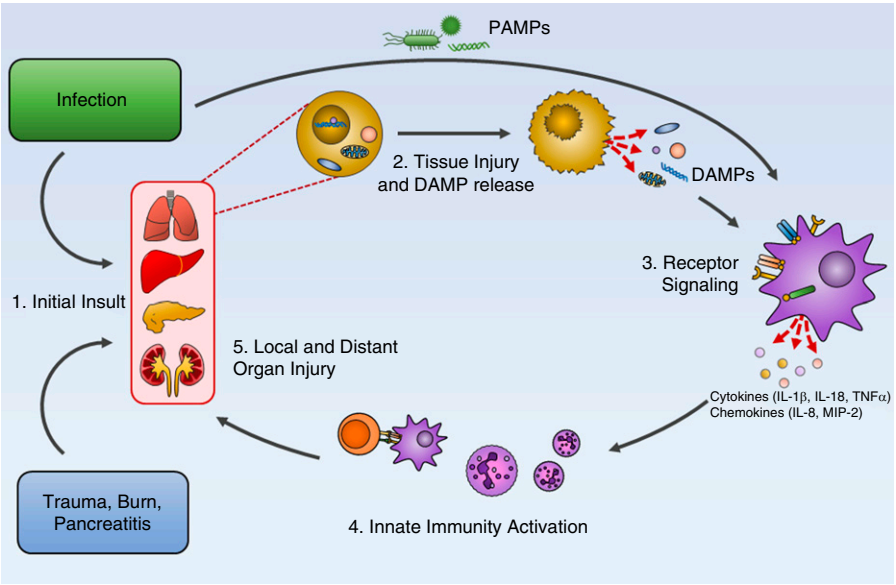

Figure 1. Proposed schema for the pathogenesis of damage-associated molecular pattern (DAMP)- and pathogen-associated molecular pattern (PAMP)-mediated local and distal organ injury during critical illness. (*1*) Infection or sterile organ injury, such as trauma, burns, or pancreatitis, leads to direct initial insult. (*2*) Infection and local tissue injury releases PAMPs and DAMPs. (*3*) PAMPs and DAMPs bind Toll-like receptors and pathogen-recognition receptors, with subsequent cytokine and chemokine release. (*4*) Activation of the innate immune system with neutrophil recruitment and T-cell activation. (*5*) Resultant local and distant organ injury. MIP = macrophage inflammatory protein.

been described in the literature as potential DAMPs, but few have met the above criteria (10, 21, 22). The most well-described and accepted DAMPs include nucleic acids, such as mitochondrial DNA (mtDNA) (22, 23) and nuclear DNA (24, 25), HMGB1 (high mobility group [HMG] box 1) (26), HSPs (heat shock proteins) (27), histones (28), and S100 proteins (29) (Table 1). Other potential DAMPs include intracellular uric acid (30), N-formyl peptides (31), defensins (32), cathelicidins (33), extracellular hyaluronic acid (34), and fibronectin (35).

Given the similarities between DAMPs and PAMPs, it should be no surprise that DAMPs also activate PRR pathways and lead to NF-κB and inflammasome activation. These cascades can, in turn, lead to local cellular necrosis, further release of DAMPs, and the propagation of dysregulated cell death (36). Specifically, DAMPs, such as HMGB1, mtDNA, S100 proteins, and histones, have been shown to activate TLRs, including TLR2 (37), TLR3 (38), TLR4 (37), and TLR9 (39). More recently, immunologically active receptors that recognize DAMPs, but not PAMPs, have been identified. For example, RAGE (the receptor for advanced glycation endproducts) is a plasma membrane receptor that has been shown to respond to a variety of DAMPs, including HMGB1 (40) and S100 proteins (41). RAGE was first discovered as a receptor for products of nonenzymatic glycation and oxidation of macromolecules (42). RAGE is found in high levels in lung tissue, but can also appear in innate immune cells, including macrophages and neutrophils. Once bound by its ligands, RAGE can activate NF-κB and mitogen-activated protein kinase pathways, leading to a proinflammatory state (43).

This review focuses on the best-described DAMPs and their role in the pathogenesis of critical illness, with a specific focus on sepsis, trauma, acute lung injury, and cardiac arrest. We also discuss current and future translational and therapeutic research in this field targeting DAMP-associated pathways in critical illness.

DAMPs and Their Role in Critical Illness

Nucleic Acids

Except for mature erythrocytes, all human cells contain nucleic acids in the form of nuclear DNA, RNA, and mtDNA. Microbial nucleic acids are well-known PAMPs (44–47). During times of cellular stress, the release of host nucleic acids into the cytosolic and extracellular space illicit an inflammatory response like that of microbial nucleic acids. *In vitro* stimulation with cell-free nuclear DNA can selectively stimulate the production of IL-6 by human monocytes (24). Similarly, mRNA has *in vitro* inflammatory activity through TLR3-dependent induction of the NK-κB pathway and IL-8 secretion (38). In human subjects, plasma nuclear DNA concentrations are higher in those undergoing treatment in a mixed ICU population compared with healthy control subjects and correlates with mortality and need for mechanical ventilation (25, 48). Compared with healthy control subjects, plasma nuclear DNA is higher in human subjects with sepsis or septic shock (49, 50). This correlation is also seen in murine models of hemorrhagic shock (51) and rate models of trauma (52). Plasma nuclear DNA concentrations are also associated with severity of trauma and the presence of acute lung injury (53).

Mitochondria are bacterial endosymbionts that have evolved to be vital organelles in cellular energy production. Mitochondria carry their own genetic code, such as mtDNA, and transcriptional machinery, allowing for production of mitochondria-specific proteins. mtDNA is also CpG enriched, like bacterial DNA. Cellular stress, such as stimulation with ATP and LPS, can release mtDNA into the cytosolic and extracellular space, leading to activation of the innate immune system through binding of TLR receptors, specifically TLR9 (22, 39). Human neutrophils treated with purified mtDNA demonstrate MMP (matrix metallopeptidase)-8 and MMP9 release. Mice injected with mitochondrial debris or purified mtDNA have higher levels of TNF-α, IL-6, and IL-1 in the plasma, higher levels of IL-6 and TNF-α in the liver, as well as increased markers of lung injury (54, 55).

Among hospital nonsurvivors, mtDNA levels are higher at presentation to the emergency department and 72 hours after admission (23, 56). mtDNA levels are higher in patients who have undergone trauma admitted to the ICU compared with healthy control subjects, and correlates with severity of the inflammatory response and the development of MODS (51, 57–60). Similarly, plasma DNA levels correlate with mortality after out-of-hospital cardiac arrest (61, 62). In a separate cohort of 85 patients, cell-free mtDNA was superior to

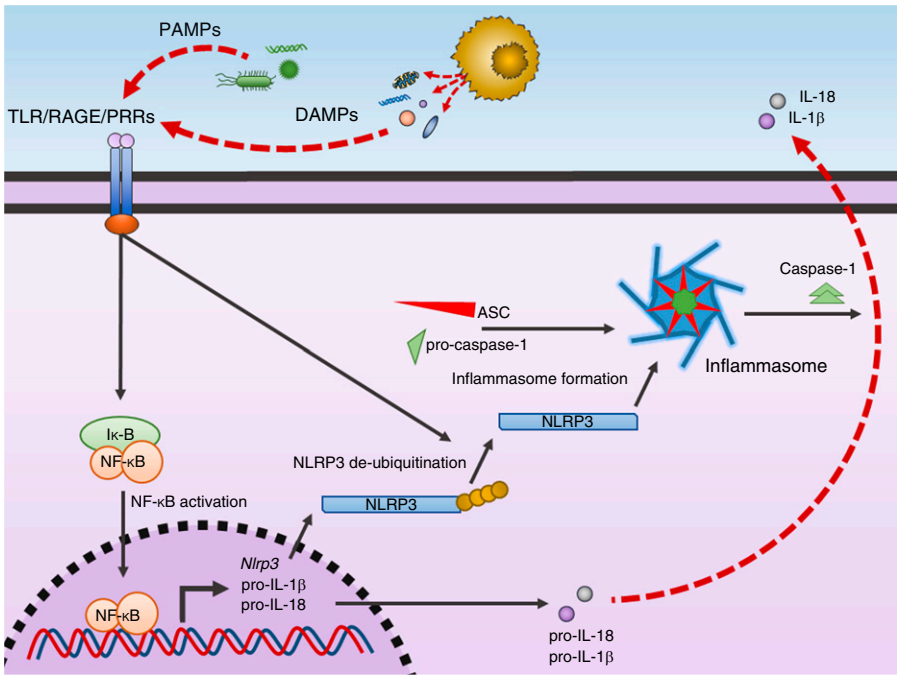

Figure 2. Schema of damage-associated molecular pattern (DAMP)- and pathogen-associated molecular pattern (PAMP)-mediated activation of innate immunity and inflammation. PAMPs and DAMPs released from infection and tissue injury, respectively, bind cell surface TLRs (Toll-like receptors) and PRRs (pattern-recognition receptors), leading to activation of NF-κB (nuclear factor-κB). NF-κB translocates to the nucleus and promotes expression of pro–IL-18, pro–IL1β, and NLRP3 (nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3). NLRP3 is deubiquinated and combines with apoptosis-associated speck-like protein (ASC) and pro–caspase-1 to form the NLRP3 inflammasome. The activated NLRP3 inflammasome converts pro–caspase-1 to caspase-1. Caspase-1 subsequently then catalyzes the production of mature IL-18 and IL-1β. Iκ-B = inhibitor of κ-B; RAGE = receptor for advanced glycation end-products.

nuclear DNA in predicting survival at Day 3 after cardiac arrest (63).

HMGB1

HMGB1 is a member of the HMG family of proteins that are present in numerous eukaryotic organisms. HMG proteins reside within the nucleus and regulate transcription as a DNA chaperone (64). HMGB1 is released in response to cellular stress (10). Administration of purified HMGB1 activates the innate immune system (65), whereas inactivation of HMGB1 through antibodies or siRNA suppresses the inflammatory response (66). HMGB1 was first noted to be a potential late mediator of inflammation in 1999 by Wang and colleagues (10). Secretion of HMGB1 occurs 6–8 hours after LPS exposure, significantly later than TNF-α or IL-1. Release of HMGB1 extracellularly interacts with receptors, including TLR2, TLR4, TLR9, and RAGE (67–69), to release inflammatory cytokines, including TNF-α, IL-1, and IL-6 (10, 70).

HMGB1 was found to be elevated beginning 18 hours after cecal ligation and puncture (CLP) in mice, and remains elevated for up to 4 weeks (66, 71). Injections of anti-HMGB1 antibodies were able to ameliorate CLP-induced mortality (66). In humans, HMGB1 is elevated in patients with sepsis and septic shock, and is associated higher mortality (10, 72, 73). Compared with sepsis, traumatic injury is associated with a more rapid release of HMGB1 (74). In a murine model of hemorrhagic shock, treatment with anti-HMGB1 antibody improved survival at 24 hours after insult compared with control. In this same study, a human cohort of 25 patients admitted for trauma and hemorrhagic shock demonstrated peak plasma HMGB1 level within 6 hours of injury that remained elevated for at least 24 hours (74). In other studies, plasma HMGB1 is elevated within 30 minutes after trauma, and is associated with severity of injury and subsequent development of sepsis, MODS, and death (75–77). Elevated HMGB1 level has also been associated with patients requiring prolonged mechanical ventilation (78), as well as poor outcomes after cardiac arrest (79).

HSPs

HSPs are ubiquitous molecular chaperone proteins conserved across virtually all species. During normal cellular homeostasis, HSPs bind polypeptide chains to prevent protein aggregation and misfolding (80). The HSP70 family of at least 13 distinct human proteins is synthesized and released under a variety of environmental and pathological stresses, including infection (81), trauma (82), and hyperthermia (83). However, unlike HMGB1 or mtDNA, extracellular members of the HSP70 family can exhibit either inflammatory or antiinflammatory properties. T regulatory cells treated with purified HSP72, an endotoxin-inducible HSP70 family member, have decreased secretion of proinflammatory cytokines, such as IFNγ and TNF-α, and an increased secretion of antiinflammatory cytokines, such as IL-10 (84). *In vitro* treatment with HSP72 decreased activation of allogeneic T cells by immature dendritic cells (85).

Induction of several HSP70 family members through different methods was protective against animal models of sepsis (86–89). Aged transgenic *hsp70*$^{-/-}$ mice, which are deficient in the homolog for human HSP72, had higher mortality compared with wild-type mice subjected to CLP, *Pseudomonas aeruginosa* pneumonia, and *Streptococcus pneumoniae* pneumonia. In addition, these mice had higher levels of systemic inflammatory cytokines, including TNF-α, IL-6, IL-10, and IL-1β (90). In humans, HSPs are upregulated in adults (91, 92) and children (93) with sepsis. Polymorphisms in *hsp70A1A*, *-A1B*, and *-A1L* may also be correlated with increased morbidity after sepsis (94). In models of hemorrhage and resuscitation, mice have higher levels of HSP70 in the jejunum, lung, heart, kidney, and liver compared with sham controls (95). In a cohort of 67 patients admitted with trauma, HSP72 levels were significantly higher in those with injury severity scores greater than 16 compared with those with scores less than 16 and healthy control subjects. However, among patients admitted with severe trauma, higher HSP72 values at time of admission were associated with improved survival (96). Similarly, HSP72 is increased immediately after cardiac arrest and remains elevated over the following day (97). HSP72 levels are

Table 1. Five Commonly Reported Damage-associated Molecular Patterns, Their Reported Receptors, Studies of Preclinical Models of Critical Illness, and Studies of Human Cohorts of Critical Illness

DAMP	Receptor(s)	Nonhuman Studies	Human Studies
Mitochondrial DNA	TLR9 (39) and AIM2 (22)	Sepsis (22) Trauma (52) ALI (52, 55) Shock (153) Burn (154)	Sepsis (23, 56) Trauma (51, 57–60) Cardiac arrest (63)
Nuclear DNA	TLR9 (39)	Sepsis (138) Trauma (52)	Sepsis (49, 50) Trauma (53) Cardiac arrest (61, 62) Burn (155)
HMGB1	TLR2 (67), TLR4 (67, 156), TLR9 (69), and RAGE (10)	Sepsis (66, 71) Trauma (74) ALI (156, 157) Cardiac arrest (158) Burn (159)	Sepsis (10, 72, 73) Trauma (74–77) VILI (78) Cardiac arrest (79, 160) Burns (161)
Heat shock proteins	TLR2 (162), TLR4 (162), SREC-1 (163), CD91 (164), and CD14 (165)	Sepsis (86–89) Trauma (166) Shock (95) ALI (167)	Sepsis (91–93) Trauma (96) Cardiac arrest (97) ALI (168)
S100 Proteins	TLR4 (100, 101) and RAGE (41)	Sepsis (107, 108) Trauma (103, 104) ALI (169)	Sepsis (109, 110) Trauma (111, 112) ALI (113–115) Cardiac arrest (18, 116, 117)
Histones	TLR2 (120, 121), TLR4 (120, 121),TLR9 (119), and NLPR3 (122)	Sepsis (124) Trauma (123) ALI (123)	Sepsis (125, 126) Trauma (123) ALI (123) Cardiac arrest (170)

Definition of abbreviations: AIM2 = absent in melanoma 2; ALI = acute lung injury; DAMP = damage-associated molecular pattern; HMGB1 = high-mobility group box 1; NLPR3 = nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3; RAGE = receptor for advanced glycation end-products; SREC-1 = scavenger receptor expressed by endothelial cells I; TLR = Toll-like receptor; VILI = ventilator-induced lung injury.

negatively correlated with proinflammatory cytokines, such as TNF-α and IL-6 (97).

S100 Proteins

S100 proteins are a family of more than 20 proteins seen exclusively within vertebrates. S100 proteins typically form homodimers that are capable of binding intracellular calcium and target proteins, serving as a calcium sensor for the regulation of effector proteins (98). Three specific S100 proteins (S100A8, S100A9, and S100A12) have been found to be specific to myeloid cells and highly regulated during the inflammatory process (29). S100A8, also known as myeloid-related protein 8 and S100A9, also known as myeloid-related protein 14, are present in circulatory granulocytes, but not tissue macrophages (29). During inflammation, the two proteins form a heterodimer and are released into the extracellular space (99). S100A8/S100A9 heterodimers regulate the inflammatory response through ICAM-1 (intercellular adhesion molecule 1)–mediated neutrophil chemotaxis and TLR4-mediated cytokine release (29, 100, 101). S100A12 is a more recently described S100 protein that activates the inflammatory axis through binding of either RAGE or TLR4 (41, 102). When released into the extracellular space, S100B can act as a ligand for RAGE-mediated signaling and NF-κB activation. S100B has been studied extensively in neurologic illness, and has been shown to be altered in numerous forms of brain injury, including trauma (103, 104), anoxia (105), and hemorrhage (106).

In sepsis models, S100A8/S100A9 is upregulated in the lung and plasma in mice during bacterial pneumonia. However, conflicting reports suggest that S100A8/S100A9 may be protective against bacterial dissemination and sepsis-associated mortality during infection with *Klebsiella pneumoniae*, but detrimental during infection with pneumococcal pneumonia (107, 108). Mice deficient in S100A9 are protected from endotoxin-induced lethal shock and *Escherichia coli*–induced abdominal sepsis (101). In humans, patients with septic shock who died had higher mRNA expression of S100A8 compared with survivors (109). S100A12 levels in the serum were higher in patients with sepsis from peritonitis, pneumonia, or urinary tract infection compared with healthy control subjects (110). After trauma, S100A8 and S100A12 are elevated, and reach their peak between 4 and 6 days after trauma (111). S100A8/S100A9 is also significantly higher in patients who have undergone trauma and who died on Day 1 of admission compared with survivors (112). S100A12 is elevated in the BAL fluid of subjects with acute respiratory distress syndrome compared with healthy control subjects (113–115). Significantly elevated levels of S100B after cardiac arrest have been associated with poor neurologic outcomes (18, 116) and lower rates of survival (117).

Histones

Histones are intranuclear proteins that bind to DNA to enhance chromatin stability

and allow for epigenetic regulation of DNA (28). Within the nucleus, histones have two major functions. Core histones compress DNA by wrapping 147 bp of DNA around an octameric core histone complex, forming the "nucleosome." Linker histones then combine multiple nucleosomes together, forming chromatin (28). Both nucleosomes and free histones can be released into the extracellular space after unregulated necrotic cell death (118). When histones are released into the extracellular space, they target proinflammatory receptors, including TLR2, TLR4, and TLR9, as well as direct activation of the NLRP3 inflammasome (119–122). They also directly induce cell death through disruption of plasma membranes and subsequent calcium influx (123).

Administration of anti-histone antibodies reduced the mortality of mice in LPS, TNF-α, and CLP models of sepsis (124). Circulating histones are elevated in patients with sepsis compared with healthy control subjects (125, 126). In a cohort of 65 patients with sepsis, high histone levels were correlated with degree of organ failure, new-onset left ventricular dysfunction, and mortality (125). Administration of exogenous histones to mice produced similar levels of lung injury and coagulation abnormalities as those produced by trauma. Coadministration of anti-histone antibodies, or concurrent treatment with anti-histone antibodies 10 minutes before trauma, ameliorated these effects (123). Patients with trauma had elevated levels of circulating nucleosomes compared with healthy control subjects, and levels of circulating nucleosomes after trauma were correlated with development of post-traumatic respiratory failure (123). In another study of different ICU patients, extracellular plasma histone H4 (a core histone) concentration was most elevated in patients with multiple trauma as well as sepsis compared with ICU control subjects. Higher histone H4 concentration was also associated with need for renal replacement therapy and 90-day mortality (126).

Current Controversies and Unexplored Areas

Differential Expression of DAMPs during Critical Illness

Despite a robust body of preclinical studies demonstrating the effects of individual DAMPs on innate immunity and inflammation, no studies have elucidated precise stimuli or triggers that result in a differential expression of DAMPs. In a therapeutic study of intensive glycemic control using insulin in surgical ICU patients, HMGB1 and S100A12 were similarly elevated in both cohorts (127). In a cohort of patients with trauma in the emergency department, nuclear DNA and HSP70, but not mtDNA, were elevated compared with healthy control subjects (82). However, these findings contradict other studies of plasma mtDNA in patients with trauma, which show that mtDNA is elevated after trauma and is correlated to severity of injury and outcomes (51, 57–59). The lack of differential expression in the majority of published reports is not necessarily surprising. DAMPs are intracellular molecules that are typically passively released in the setting of cell stress and death, a feature common in many critical care states (128–130).

Mechanisms of Active DAMP Release

Although DAMPs are typically passively released in the setting of cell death, growing evidence suggests that certain DAMPs may also be actively secreted into the extracellular space. Monocytes and macrophages exposed to LPS or TNF-α can acetylate or phosphorylate the nuclear localization signals of HMGB1, leading to relocation of HMGB1 from the nucleus into the cytoplasmic space (131, 132). HMGB1 is then packaged into secretory lysosomes, where they can then be excreted into the extracellular space in response to triggers, such as lysophosphatidylcholine (133). HSPs have been shown to be trafficked to the cell surface via exosomes after stimulation with IFNγ or heat shock, a mechanism that is independent of the common protein-secretory pathway (134–136). Using time-lapse automated confocal imaging, Yousefi and colleagues (137) demonstrated that eosinophils released mtDNA, but not nuclear DNA, in a "catapult-like" manner after exposure to LPS, complement factor 5a, or eotaxin. The precise mechanism of this release is unclear, but appears to require activation of nicotinamide adenine dinucleotide phosphate reduced oxidase, but is independent of cell death (137). Whether these specific release mechanisms respond to all external environmental stressors or are trigger specific remains unknown. Further studies are also required to assess whether these lysosome- and exosome-dependent secretory pathways are shared among DAMPs or are specific for certain DAMPs.

Modulation of DAMP Expression or Release during Critical Illness

Preclinical studies exploring the modulation of DAMPs during critical illness have often focused on either the neutralization of DAMPs with antibodies or pharmacologic intervention before or after onset of critical illness. Use of anti-HMGB1 antibody has been shown to improve survival in murine models of sepsis and hemorrhagic shock (66, 74). The same group has also reported that (−)-epigallocatechin-3-gallate (EGCG), an ingredient in green tea, suppressed LPS-induced release of HMGB1 from macrophages, and rescued mice from lethal sepsis after CLP. EGCG also inhibited HMGB1-mediated release of inflammatory cytokines by blocking aggregation of exogenous HMGB1 on the surface of macrophages. No other DAMPs were evaluated, so it is unclear whether the beneficial effects of EGCG are specific to HMGB1 or applicable to other DAMPs.

Mice that are treated with DNase 4 or 6 hours after CLP have reduced cell-free DNA, suppressed organ damage, and reduced bacterial dissemination (138). Similarly, intratracheal DNase administration to rat lungs before or after intratracheal infection with *P. aeruginosa* mitigated endothelial injury and mtDNA in the BAL (139). Thus far, no human studies have directly inhibited DAMP release or binding. Interestingly, some of the antiinflammatory effects of HSP72 may be related its modulation of HMGB1 release. In macrophages, release of HSP72 with mild heat shock or overexpression via gene transfection leads to inhibition of HMGB1 cytoplasmic translocation and release after oxidative stress, LPS, and TNF-α treatment (140, 141).

Modulation of DAMP–Receptor Interaction during Critical Illness

Investigators have also explored the possibility of modulating DAMP signaling through the blocking of common DAMP receptors, including TLR4 and RAGE. TAK-242 (resatorvid) is a small-molecule–specific inhibitor of TLR4 signaling through binding of the

intracellular domain of TLR4. In mice, administration of TAK-242 before or after LPS challenge suppressed expression of inflammatory cytokines, including TNF-α, IL-1β, and IL-6. Mice treated with TAK-242 before or after LPS challenge also demonstrated lower markers of organ injury, such as alanine aminotransferase and total bilirubin (142). However, a multicenter, randomized, double-blind, placebo-controlled trial of TAK-242 in 274 patients with severe sepsis and shock or respiratory failure was stopped early due to lack of efficacy in suppressing serum IL-6 levels (143).

E5564 (eritoran) is a synthetic LPS derived from the endotoxin of *Rhodobacter sphaeroides*. Eritoran blocks LPS-mediated cytokine release through the binding of an internal pocket of MD2, a protein necessary to facilitate the binding of LPS to TL4 (144). Phase I clinical studies demonstrated reductions in vital sign changes as well as decreased expression of TNF-α and IL-6 after treatment with eritoran in healthy subjects challenged with LPS (145). However, follow-up phase II and phase III trials failed to demonstrate a difference in 28-day mortality after treatment with eritoran among patients with severe sepsis and organ dysfunction (146, 147).

van Zoelen and colleagues (148) have previously demonstrated that RAGE-deficient mice show improved survival after intranasal inoculation with *S. pneumoniae* compared with wild-type mice. Similarly, the use of an anti-RAGE monoclonal antibody was protective in murine sepsis with both CLP and *S. pneumoniae* inoculation (149, 150). RAGE signaling has been associated with increased lethality in mice exposed to CLP or *S. pneumoniae* inoculation (148, 150). However, more recently van Zoelen and colleagues have also shown that RAGE is protective during *K. pneumoniae*–induced pneumonia. Thus, current work on RAGE has yielded inconsistent results and remains preclinical. These studies highlight the complexity of treating human disease when effective therapies observed in preclinical disease models sometimes (oftentimes) are not replicated in human diseases.

Future Directions

As highlighted in this review, systemic and tissue-specific levels of various DAMPs are associated with progression of key critical illnesses. From the basic and early translational research performed in recent decades, the importance of these cellular breakdown products in inflammatory signal transduction has become clear. In the near term, several best-studied DAMPs, such as nucleic acids, may play a role in prognostication in the ICU. However, the focus on DAMPs research should not focus on its prognostic significance in human cohorts, but more on the precise mechanisms of DAMP-induced inflammation and cell injury. In many conditions encountered in the ICU, multiple DAMPs may be crucial to the development of a specific response. Prior work has focused on individual markers and interaction with specific PRRs. In most clinical situations, there are complex, and likely redundant, interactions between different DAMP–PRR pathways. Moreover, differential levels and patterns of DAMP kinetics may play an important role in the diagnosis of syndromic disease states, such as sterile systemic inflammatory response syndrome and ongoing occult infection. There is much work to be done to understand stimulus-specific differential DAMP responses. Likely, there is a complex interplay between host genomic and metabolic parameters and DAMP-specific response to certain stimuli that will vary in different patient populations, and this needs further elucidation. Further understanding of the feedback loops that can propagate DAMP release and ongoing cell death will be important in designing clinical interventions in these conditions. Recent work has highlighted the importance of necroptotic cell death in the immunologically active release of various cellular components, many of which are DAMPs (151). Truly understanding when and where in the host these pathways are appropriately activated versus dysregulated will be key to appropriately targeting inhibitors.

Conclusions

Given the past failures of antiinflammatory therapy, it is necessary to move beyond animal and cell culture models of DAMPs. Unfortunately, there is no "time zero" in many ICU diseases, and the time course of the immune response to insult is often not known. Humans are more complex than most laboratory animals, carrying immune histories and comorbidities over a long and varied life span. Importantly, robust real-world translational data are needed regarding DAMP, PRR, and pathway propagation, such as the important contribution of Nakahira and colleagues (22). Deep understanding of subphenotypes and differential individual time-dependent phases of classic syndromic disease states, such as sepsis and trauma, which characterize the innate immune system response, will be integral (152). Taking these data, and understanding the dysregulated cell death pathway activation through a combination of clinical and molecular methods, will be important to designing adaptive, personalized clinical trials with a high likelihood of finding targeted therapies. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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