AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

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

Authors: Kevin C. Ma[1], Edward J. Schenck[1], Maria A. Pabon[2], Augustine M.K. Choi[1],

[1]Division of Pulmonary and Critical Care Medicine, Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medicine, New York, NY

[2]Division of General Internal Medicine, Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medicine, New York, NY

All work performed at NewYork-Presbyterian Hospital/Weill Cornell Medicine

Corresponding Author: Augustine M.K. Choi, 1300 York Avenue, Suite F-113 Box 83, New York, New York 10065, Telephone: 212-746-6005 email: amc2056@med.cornell.edu

Statement of Authorship: KCM, AMKC, EJS contributed to the conception and design of the study; KCM, EJS , MAP contributed to the drafting of the manuscript; KCM, AMKC, EJS contributing to the editing of manuscript; KCM contributed to the creation of the figures; AMKC and EJS contributed to the editing of the figures. All authors contributed to the final approval of all submitted contents

Funding Information: Supported by National Institutes of Health grants P01 HL108801, R01 HL079904, R01 HL055330 and R01 HL060234 (A.M.K.C.), Supported by National Institutes of Health grant KL2TR000458-10 (E.J.S.)

Running head: Danger Signals in the Pathogenesis of Critical Illness

Descriptor Number: 4.9 Molecular Biology of Critical Care OR 4.12 Sepsis/Multiple Organ Failure

Word Count:

Body: 3815

Abstract: 250

Abstract

The innate immune system induces inflammation through induction of multiple pathways including the TNFα/NF-κB signaling as well as the NLRP3 inflammasome cascade activation with production of inflammatory molecules. Pathogen associated molecular patterns are immunogenic costimulatory molecules that can activate the innate immune system in the presence of foreign pathogens. These molecularly distinct, foreign, particles initiate inflammatory and cell death cascades of the innate immune response through the binding of pathogen recognition receptors such as toll like receptors. The concept of danger associated molecular patterns was proposed in the 1990s based on observations that noninfectious critical illness produces similar inflammatory reactions. Endogenous danger signals are commonly intracellular molecules, such as nuclear or mitochondrial nucleic acids, that have individual biologic roles in non-inflammatory states. However, once released into the extracellular space, these molecular patterns stimulate the innate immune response through binding of similar pathogen recognition receptors. Excessive release of danger signals during critical illness may create a self-perpetuating cycle of dysregulated inflammation, cell death, and subsequent distal organ injury. Danger signals have been correlated with numerous clinical outcomes in a variety of critical illnesses including sepsis, trauma, ventilator induced lung injury, and cardiac arrest. Here we highlight the most commonly reported endogenous danger signals in both animal and human studies and their potential role in the morbidity and mortality in the intensive care unit. In addition, we discuss current unanswered questions including the elucidation of downstream signaling cascades as well as therapeutic trials modulating the innate immune response through danger signal pathways.

Word count: 250/250

Key Words: Alarmins, DAMPs, Critical Illness, Sepsis

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

The Stranger and Danger models:

In 1989, Janeway introduced a conceptual framework to understand how the innate immune system selectively responds to potentially threatening infections (1). 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 lipopolysaccharide (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 pattern recognition receptors (PRRs) (4, 5). This broad class of receptors, typified by the Toll Like Receptor (TLR) 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 TNFα/NF-κB signaling cascade as well as NLRP3 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 IFNɣ (7).

This framework, however, fails to explain the systemic inflammatory response observed during non-infectious 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 1990’s 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 (SIRS) and perpetuation of the multi-organ 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), drug-induced liver injury (17), and linked to outcomes after cardiac arrest (18).

In order 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, 5. Have a separate biological role in non-inflammatory states. Since the proposal of the initial "Danger" hypothesis, numerous host-derived intracellular and extracellular molecules have 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), high-mobility group box 1 (HMGB1) (26), heat shock proteins (HSPs) (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

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

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 toll-like receptors 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, the receptor for advanced glycation endproducts (RAGE) 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 non-enzymatic 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 (MAPK) pathways leading to a proinflammatory state (43).

This review will focus 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 will 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 mitochondrial DNA. 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, messenger RNA 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 to healthy controls and correlates with mortality and need for mechanical ventilation (25, 48). Compared to healthy controls, 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, 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 MMP8 and MMP9 release. Mice injected with mitochondrial debris or purified mtDNA have higher levels of TNFα, IL-6, and IL-1 in the plasma, higher levels of IL-6 and TNFα in the liver as well as increased markers of lung injury (54, 55).

Among hospital non-survivors, mtDNA levels are higher at presentation to the ED and 72 hours after admission (23, 56). MtDNA levels are higher in trauma patients admitted to the ICU compared to healthy controls 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 mitochondrial DNA was superior to nuclear DNA in predicting survival at day 3 after cardiac arrest (63).

High-Mobility Group Box 1 (HMGB1)

HMGB1 is a member of the high-mobility group (HMG) family of proteins that are present in numerous eukaryotic organisms. HMG proteins reside within the nucleus and regulate transcription as a

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

DNA chaperone (64). HMGB1 is released in response to cellular stress (10). Administration of purified HMGB1 activates the innate immune system (65), while 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 to 8 hours after LPS exposure, significantly later than TNFα or IL-1. Release of HMGB1 extracellularly interacts with receptors including TLR2, TLR4, TLR9, and RAGE (67–69) to release inflammatory cytokines including TNFα, 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 to 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 to 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 levels has also been associated with patients requiring prolonged mechanical ventilation (78) as well as poor outcomes after cardiac arrest (79).

Heat Shock Proteins (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 are 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 anti-inflammatory properties. T-regulatory cells treated with purified HSP72, an endotoxin inducible HSP70 family member, have decreased secretion of pro-inflammatory cytokines such as IFNɣ and TNFα, and an increased secretion of anti-inflammatory 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 homologue for human HSP72, had higher mortality compared to wildtype mice subjected to CLP, *Pseudomonas aeruginosa* pneumonia, and *Streptococcus pneumoniae* pneumonia. Additionally, these mice had higher levels of systemic inflammatory cytokines including TNFα, 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 to sham controls (95). In a cohort of 67 patients admitted with trauma, HSP72 levels were significantly higher in those with injury severity scores >16 compared to those with scores <16 and healthy controls. Yet, among patients admitted with severe trauma, higher HSP72 values at time of admission was associated with improved survival (96). Similarly, HSP72 is increased immediately after cardiac arrest and remains elevated over the following day (97). HSP72 levels are negatively correlated with proinflammatory cytokines such as TNFα and IL-6 (97).

S100 Proteins

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

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 (MRP8) and S100A9, also known as myeloid-related protein 14 (MRP14), 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 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 brain injury including trauma (103, 104), anoxia (105), or hemorrhage (106).

In sepsis models, S100A8/S100A9 is upregulated in the lung and plasma in mice during bacterial pneumonia. However, conflicting reports suggest 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 to survivors (109). S100A12 levels in the serum were higher in patients with sepsis from peritonitis, pneumonia, or urinary tract infection compared to healthy controls (110). After trauma, S100A8 and S100A12 are elevated and reach their peak between 4 to 6 days after trauma (111). S100A8/S100A9 is also significantly higher in trauma patients who died on day 1 of admission compared to survivors (112).

S100A12 is elevated in the bronchoalveolar lavage fluid of subjects with ARDS compared to healthy controls (113–115). Significantly elevated levels of S100B after cardiac arrest has 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 base pairs 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 extracelluar space, they target pro-inflammatory 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 ant-histone antibodies reduced the mortality of mice in LPS, TNFα or CLP models of sepsis (124). Circulating histones are elevated in patients with sepsis compared to healthy controls (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 trauma. Coadministration of anti-histone antibodies, or concurrent treatment with anti-histone antibodies 10 minutes prior to trauma, ameliorated these effects (123). Patients with trauma had elevated levels of circulating nucleosomes compared to healthy control subjects and levels of circulating nucleosomes after trauma was correlated with development of post-traumatic respiratory failure (123). In another study of different ICU patients, extracellular plasma histone H4 (a core histone) concentration

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

was most elevated in patients with multiple trauma as well as sepsis compared to ICU controls. 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 trauma patients in the emergency department, nuclear DNA and HSP70, but not mtDNA, were elevated compared to healthy controls (82). However, these findings contradict other studies of plasma mtDNA in trauma patients, which shows 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

While DAMPs are typically passively released in the setting of cell death, growing evidence suggest certain DAMPs may also be actively secreted into the extracellular space. Monocytes and macrophages exposed to LPS or TNFα 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 IFNɣ or heat shock, a mechanism that is independent of the common protein secretory pathway (134–136). Using time-lapse automated confocal imaging, Yousefi *et al.* demonstrated that eosinophils released mtDNA, but not nuclear DNA, in a "catapult-like" manner following exposure to LPS, complement factor 5a, or eotaxin (137). The precise mechanism of this release is unclear but appears to require activation of NADPH 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 EGCG's beneficial effects are specific to HMGB1 or applicable to other DAMPs.

Mice who are treated with deoxyribonuclease (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 *Pseudomonas aeruginosa* mitigated endothelial injury and mtDNA in the bronchoalveolar lavage (139). Thus far, no human studies have directly inhibited DAMP release or binding. Interestingly, some of the anti-inflammatory effects of

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

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 TNFα 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 TLR4's intracellular domain. In mice, administration of TAK-242 before or after LPS challenge suppressed expression of inflammatory cytokines including TNFα, 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 lipopolysaccharide 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 TNFα and IL-6 after treatment with eritoran in healthy subjects challenged with LPS (145). However, followup 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 have previously demonstrated that RAGE deficient mice show improved survival after intranasal inoculation with *S. pneumoniae* compared to wildtype mice (148). 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 *Klebsiella pneumoniae* induced pneumonia. Thus, current work on RAGE has yielded inconsistent results and remain preclinical. These studies highlight the complexity of treating human disease when effective therapies observed in preclinical disease models sometimes (often times) 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 intensive care unit, 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 SIRS 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

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

active release of many cellular components, many of which are DAMPs (151). Truly understanding when and where in the host these pathways are appropriately activated vs dysregulated will be key to appropriately targeting inhibitors.

Conclusions

Given the past failures of anti-inflammatory 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 is needed regarding DAMP, PRR and pathway propagation, such as the important contribution of Nakahira *et al* (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 this 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.

References

1. Janeway CA. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol* 1989;54 Pt 1:1–13.
2. Dresser DW. Effectiveness of lipid and lipidophilic substances as adjuvants. *Nature* 1961;191:1169–1171.
3. Janeway CA, Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002;20:197–216.
4. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001;2:675–680.
5. Medzhitov R, Janeway CA. Innate immunity: the virtues of a nonclonal system of recognition. *Cell* 1997;91:295–298.
6. Palm NW, Medzhitov R. Pattern recognition receptors and control of adaptive immunity. *Immunol Rev* 2009;227:221–233.
7. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin Microbiol Rev* 2009;22:240–273, Table of Contents.
8. Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994;12:991–1045.
9. Matzinger P. The Danger Model: A Renewed Sense of Self. *Science* 2002;296:301–305.
10. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999;285:248–251.
11. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002;418:191–195.
12. Goldberg B, Urnovitz HB, Stricker RB. Beyond danger: unmethylated CpG dinucleotides and the immunopathogenesis of disease. *Immunol Lett* 2000;73:13–18.

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

13. Denk S, Perl M, Huber-Lang M. Damage- and Pathogen-Associated Molecular Patterns and Alarmins: Keys to Sepsis. *Eur Surg Res* 2012;48:171–179.

14. Kang J-W, Kim S-J, Cho H-I, Lee S-M. DAMPs activating innate immune responses in sepsis. *Ageing Res Rev* 2015;24:54–65.

15. Hirsiger S, Simmen H-P, Werner CML, Wanner GA, Rittirsch D. Danger Signals Activating the Immune Response after Trauma. *Mediators Inflamm* 2012;2012:.

16. Kuipers MT, van der Poll T, Schultz MJ, Wieland CW. Bench-to-bedside review: Damage-associated molecular patterns in the onset of ventilator-induced lung injury. *Crit Care* 2011;15:235.

17. Marques PE, Amaral SS, Pires DA, Nogueira LL, Soriani FM, Lima BHF, Lopes GAO, Russo RC, Avila TV, Melgaço JG, Oliveira AG, Pinto MA, Lima CX, De Paula AM, Cara DC, Leite MF, Teixeira MM, Menezes GB. Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure. *Hepatology* 2012;56:1971–1982.

18. Shinozaki K, Oda S, Sadahiro T, Nakamura M, Hirayama Y, Abe R, Tateishi Y, Hattori N, Shimada T, Hirasawa H. S-100B and neuron-specific enolase as predictors of neurological outcome in patients after cardiac arrest and return of spontaneous circulation: a systematic review. *Crit Care* 2009;13:R121.

19. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* 2007;81:1–5.

20. Oppenheim JJ, Yang D. Alarmins: chemotactic activators of immune responses. *Curr Opin Immunol* 2005;17:359–365.

21. Nakahira K, Hisata S, Choi AMK. The Roles of Mitochondrial Damage-Associated Molecular Patterns in Diseases. *Antioxid Redox Signal* 2015;23:1329–1350.

22. Nakahira K, Haspel JA, Rathinam VAK, Lee S-J, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, Kim HP, Fitzgerald KA, Ryter SW, Choi AMK. Autophagy proteins regulate innate

immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat Immunol* 2011;12:222–230.

23. Nakahira K, Kyung S-Y, Rogers AJ, Gazourian L, Youn S, Massaro AF, Quintana C, Osorio JC, Wang Z, Zhao Y, Lawler LA, Christie JD, Meyer NJ, Causland FRM, Waikar SS, Waxman AB, Chung RT, Bueno R, Rosas IO, Fredenburgh LE, Baron RM, Christiani DC, Hunninghake GM, Choi AMK. Circulating Mitochondrial DNA in Patients in the ICU as a Marker of Mortality: Derivation and Validation. *PLOS Med* 2013;10:e1001577.
24. Atamaniuk J, Kopecky C, Skoupy S, Säemann MD, Weichhart T. Apoptotic cell-free DNA promotes inflammation in haemodialysis patients. *Nephrol Dial Transplant* 2012;27:902–905.
25. Wijeratne S, Butt A, Burns S, Sherwood K, Boyd O, Swaminathan R. Cell-free plasma DNA as a prognostic marker in intensive treatment unit patients. *Ann N Y Acad Sci* 2004;1022:232–238.
26. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* 2005;5:331–342.
27. Wallin RPA, Lundqvist A, Moré SH, von Bonin A, Kiessling R, Ljunggren H-G. Heat-shock proteins as activators of the innate immune system. *Trends Immunol* 2002;23:130–135.
28. Silk E, Zhao H, Weng H, Ma D. The role of extracellular histone in organ injury. *Cell Death Dis* 2017;8:e2812.
29. Ryckman C, Vandal K, Rouleau P, Talbot M, Tessier PA. Proinflammatory Activities of S100: Proteins S100A8, S100A9, and S100A8/A9 Induce Neutrophil Chemotaxis and Adhesion. *J Immunol* 2003;170:3233–3242.
30. Kono H, Chen C-J, Ontiveros F, Rock KL. Uric acid promotes an acute inflammatory response to sterile cell death in mice. *J Clin Invest* 2010;120:1939–1949.

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

31. Hazeldine J, Hampson P, Opoku FA, Foster M, Lord JM. N-Formyl peptides drive mitochondrial damage associated molecular pattern induced neutrophil activation through ERK1/2 and P38 MAP kinase signalling pathways. *Injury* 2015;46:975–984.

32. Xie G-H, Chen Q-X, Cheng B-L, Fang X-M. Defensins and sepsis. *BioMed Res Int* 2014;2014:180109.

33. Kościuczuk EM, Lisowski P, Jarczak J, Strzałkowska N, Jóźwik A, Horbańczuk J, Krzyżewski J, Zwierzchowski L, Bagnicka E. Cathelicidins: family of antimicrobial peptides. A review. *Mol Biol Rep* 2012;39:10957–10970.

34. Nobili V, Alisi A, Torre G, De Vito R, Pietrobattista A, Morino G, De Ville De Goyet J, Bedogni G, Pinzani M. Hyaluronic acid predicts hepatic fibrosis in children with nonalcoholic fatty liver disease. *Transl Res J Lab Clin Med* 2010;156:229–234.

35. Ruiz Martín G, Prieto Prieto J, Veiga de Cabo J, Gomez Lus L, Barberán J, González Landa JM, Fernández C. Plasma fibronectin as a marker of sepsis. *Int J Infect Dis* 2004;8:236–243.

36. Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol* 2010;10:826–837.

37. Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ, Fenton MJ, Tracey KJ, Yang H. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. *Shock Augusta Ga* 2006;26:174–179.

38. Karikó K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA is an endogenous ligand for Toll-like receptor 3. *J Biol Chem* 2004;279:12542–12550.

39. Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, Chen S, Ramanujan VK, Wolf AJ, Vergnes L, Ojcius DM, Rentsendorj A, Vargas M, Guerrero C, Wang Y, Fitzgerald KA, Underhill DM, Town T, Arditi M. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* 2012;36:401–414.

40. Tian J, Avalos AM, Mao S-Y, Chen B, Senthil K, Wu H, Parroche P, Drabic S, Golenbock D, Sirois C, Hua J, An LL, Audoly L, La Rosa G, Bierhaus A, Naworth P, Marshak-Rothstein A, Crow MK,

Fitzgerald KA, Latz E, Kiener PA, Coyle AJ. Toll-like receptor 9–dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat Immunol* 2007;8:487–496.

41. Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, Avila C, Kambham N, Bierhaus A, Nawroth P, Neurath MF, Slattery T, Beach D, McClary J, Nagashima M, Morser J, Stern D, Schmidt AM. RAGE Mediates a Novel Proinflammatory Axis: A Central Cell Surface Receptor for S100/Calgranulin Polypeptides. *Cell* 1999;97:889–901.
42. van Zoelen MA, Achouiti A, van der Poll T. The role of receptor for advanced glycation endproducts (RAGE) in infection. *Crit Care* 2011;15:208.
43. Bierhaus A, Stern DM, Nawroth PP. RAGE in inflammation: a new therapeutic target? *Curr Opin Investig Drugs Lond Engl 2000* 2006;7:985–991.
44. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S. A Toll-like receptor recognizes bacterial DNA. *Nature* 2000;408:740–745.
45. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 2001;413:732–738.
46. Bauer S, Kirschning CJ, Häcker H, Redecke V, Hausmann S, Akira S, Wagner H, Lipford GB. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci* 2001;98:9237–9242.
47. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H, Bauer S. Species-Specific Recognition of Single-Stranded RNA via Toll-like Receptor 7 and 8. *Science* 2004;303:1526–1529.
48. Saukkonen K, Lakkisto P, Varpula M, Varpula T, Voipio-Pulkki L-M, Pettilä V, Pulkki K. Association of cell-free plasma DNA with hospital mortality and organ dysfunction in intensive care unit patients. *Intensive Care Med* 2007;33:1624–1627.

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

49. Rhodes A, Wort SJ, Thomas H, Collinson P, Bennett ED. Plasma DNA concentration as a predictor of mortality and sepsis in critically ill patients. *Crit Care* 2006;10:R60.

50. Saukkonen K, Lakkisto P, Pettilä V, Varpula M, Karlsson S, Ruokonen E, Pulkki K, Finnsepsis Study Group. Cell-free plasma DNA as a predictor of outcome in severe sepsis and septic shock. *Clin Chem* 2008;54:1000–1007.

51. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 2010;464:104–107.

52. Gan L, Chen X, Sun T, Li Q, Zhang R, Zhang J, Zhong J. Significance of Serum mtDNA Concentration in Lung Injury Induced by Hip Fracture: *Shock* 2015;44:52–57.

53. Lo YM, Rainer TH, Chan LY, Hjelm NM, Cocks RA. Plasma DNA as a prognostic marker in trauma patients. *Clin Chem* 2000;46:319–323.

54. Zhang Q, Itagaki K, Hauser CJ. Mitochondrial DNA is released by shock and activates neutrophils via p38 map kinase. *Shock* 2010;34:55–59.

55. Zhang J-Z, Liu Z, Liu J, Ren J-X, Sun T-S. Mitochondrial DNA induces inflammation and increases TLR9/NF-κB expression in lung tissue. *Int J Mol Med* 2014;33:817–824.

56. Kung C-T, Hsiao S-Y, Tsai T-C, Su C-M, Chang W-N, Huang C-R, Wang H-C, Lin W-C, Chang H-W, Lin Y-J, Cheng B-C, Su BY-J, Tsai N-W, Lu C-H. Plasma nuclear and mitochondrial DNA levels as predictors of outcome in severe sepsis patients in the emergency room. *J Transl Med* 2012;10:130.

57. Gu X, Yao Y, Wu G, Lv T, Luo L, Song Y. The plasma mitochondrial DNA is an independent predictor for post-traumatic systemic inflammatory response syndrome. *PloS One* 2013;8:e72834.

58. Simmons JD, Lee Y-L, Mulekar S, Kuck JL, Brevard SB, Gonzalez RP, Gillespie MN, Richards WO. Elevated levels of plasma mitochondrial DNA DAMPs are linked to clinical outcome in severely injured human subjects. *Ann Surg* 2013;258:591-596; discussion 596-598.

59. Yamanouchi S, Kudo D, Yamada M, Miyagawa N, Furukawa H, Kushimoto S. Plasma mitochondrial DNA levels in patients with trauma and severe sepsis: time course and the association with clinical status. *J Crit Care* 2013;28:1027–1031.

60. Itagaki K, Kaczmarek E, Lee YT, Tang IT, Isal B, Adibnia Y, Sandler N, Grimm MJ, Segal BH, Otterbein LE, Hauser CJ. Mitochondrial DNA released by trauma induces neutrophil extracellular traps. *PloS One* 2015;10:e0120549.

61. Arnalich F, Menéndez M, Lagos V, Ciria E, Quesada A, Codoceo R, Vazquez JJ, López-Collazo E, Montiel C. Prognostic value of cell-free plasma DNA in patients with cardiac arrest outside the hospital: an observational cohort study. *Crit Care* 2010;14:R47.

62. Gornik I, Wagner J, Gašparović V, Miličić D, Degoricija V, Skorić B, Gornik O, Lauc G. Prognostic value of cell-free DNA in plasma of out-of-hospital cardiac arrest survivors at ICU admission and 24h post-admission. *Resuscitation* 2014;85:233–237.

63. Arnalich F, Codoceo R, López-Collazo E, Montiel C. Circulating cell-free mitochondrial DNA: A better early prognostic marker in patients with out-of-hospital cardiac arrest. *Resuscitation* 2012;83:e162–e163.

64. Fink MP. Bench-to-bedside review: High-mobility group box 1 and critical illness. *Crit Care Lond Engl* 2007;11:229.

65. Andersson U, Wang H, Palmblad K, Aveberger A-C, Bloom O, Erlandsson-Harris H, Janson A, Kokkola R, Zhang M, Yang H, Tracey KJ. High Mobility Group 1 Protein (Hmg-1) Stimulates Proinflammatory Cytokine Synthesis in Human Monocytes. *J Exp Med* 2000;192:565–570.

66. Yang H, Ochani M, Li J, Qiang X, Tanovic M, Harris HE, Susarla SM, Ulloa L, Wang H, DiRaimo R, Czura CJ, Wang H, Roth J, Warren HS, Fink MP, Fenton MJ, Andersson U, Tracey KJ. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. *Proc Natl Acad Sci U S A* 2004;101:296–301.

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

67. Park JS, Svetkauskaite D, He Q, Kim J-Y, Strassheim D, Ishizaka A, Abraham E. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. *J Biol Chem* 2004;279:7370–7377.

68. Hori O, Brett J, Slattery T, Cao R, Zhang J, Chen JX, Nagashima M, Lundh ER, Vijay S, Nitecki D, Morser J, Stern D, Schmidt AM. The Receptor for Advanced Glycation End Products (RAGE) Is a Cellular Binding Site for Amphoterin MEDIATION OF NEURITE OUTGROWTH AND CO-EXPRESSION OF RAGE AND AMPHOTERIN IN THE DEVELOPING NERVOUS SYSTEM. *J Biol Chem* 1995;270:25752–25761.

69. Valdés-Ferrer SI, Rosas-Ballina M, Olofsson PS, Lu B, Dancho ME, Li J, Yang H, Pavlov VA, Chavan SS, Tracey KJ. High-mobility group box 1 mediates persistent splenocyte priming in sepsis survivors: evidence from a murine model. *Shock Augusta Ga* 2013;40:492–495.

70. Wang H, Vishnubhakat JM, Bloom O, Zhang M, Ombrellino M, Sama A, Tracey KJ. Proinflammatory cytokines (tumor necrosis factor and interleukin 1) stimulate release of high mobility group protein-1 by pituicytes. *Surgery* 1999;126:389–392.

71. Chavan SS, Huerta PT, Robbiati S, Valdes-Ferrer SI, Ochani M, Dancho M, Frankfurt M, Volpe BT, Tracey KJ, Diamond B. HMGB1 mediates cognitive impairment in sepsis survivors. *Mol Med* 2012;18:930–937.

72. Gibot S, Massin F, Cravoisy A, Barraud D, Nace L, Levy B, Bollaert P-E. High-mobility group box 1 protein plasma concentrations during septic shock. *Intensive Care Med* 2007;33:1347–1353.

73. Sundén-Cullberg J, Norrby-Teglund A, Rouhiainen A, Rauvala H, Herman G, Tracey KJ, Lee ML, Andersson J, Tokics L, Treutiger CJ. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. *Crit Care Med* 2005;33:564–573.

74. Yang R, Harada T, Mollen KP, Prince JM, Levy RM, Englert JA, Gallowitsch-Puerta M, Yang L, Yang H, Tracey KJ, Harbrecht BG, Billiar TR, Fink MP. Anti-HMGB1 Neutralizing Antibody Ameliorates Gut Barrier Dysfunction and Improves Survival after Hemorrhagic Shock. *Mol Med* 2006;12:105–114.

75. Peltz ED, Moore EE, Eckels PC, Damle SS, Tsuruta Y, Johnson JL, Sauaia A, Silliman CC, Banerjee A, Abraham E. HMGB1 IS MARKEDLY ELEVATED WITHIN 6 HOURS OF MECHANICAL TRAUMA IN HUMANS. *Shock* 2009;32:17–22.

76. Cohen MJ, Brohi K, Calfee CS, Rahn P, Chesebro BB, Christiaans SC, Carles M, Howard M, Pittet J-F. Early release of high mobility group box nuclear protein 1 after severe trauma in humans: role of injury severity and tissue hypoperfusion. *Crit Care* 2009;13:R174.

77. Wang X-W, Karki A, Zhao X-J, Xiang X-Y, Lu Z-Q. High plasma levels of high mobility group box 1 is associated with the risk of sepsis in severe blunt chest trauma patients: a prospective cohort study. *J Cardiothorac Surg* 2014;9:133.

78. van Zoelen MAD, Ishizaka A, Wolthuls EK, Choi G, van der Poll T, Schultz MJ. Pulmonary levels of high-mobility group box 1 during mechanical ventilation and ventilator-associated pneumonia. *Shock* 2008;29:441–445.

79. Omura T, Kushimoto S, Yamanouchi S, Kudo D, Miyagawa N. High-mobility group box 1 is associated with neurological outcome in patients with post-cardiac arrest syndrome after out-of-hospital cardiac arrest. *J Intensive Care* 2016;4:37.

80. Hartl FU, Hayer-Hartl M. Molecular Chaperones in the Cytosol: from Nascent Chain to Folded Protein. *Science* 2002;295:1852–1858.

81. Delogu G, Lo Bosco L, Marandola M, Famularo G, Lenti L, Ippoliti F, Signore L. Heat shock protein (HSP70) expression in septic patients. *J Crit Care* 1997;12:188–192.

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

82. Timmermans K, Kox M, Vaneker M, Berg M, John A, Laarhoven A, Hoeven H, Scheffer GJ, Pickkers P. Plasma levels of danger-associated molecular patterns are associated with immune suppression in trauma patients. *Intensive Care Med* 2016;42:551–561.

83. Yang RC, Wang CI, Chen HW, Chou FP, Lue SI, Hwang KP. Heat shock treatment decreases the mortality of sepsis in rats. *Kaohsiung J Med Sci* 1998;14:664–672.

84. Wachstein J, Tischer S, Figueiredo C, Limbourg A, Falk C, Immenschuh S, Blasczyk R, Eiz-Vesper B. HSP70 Enhances Immunosuppressive Function of CD4 + CD25 + FoxP3 + T Regulatory Cells and Cytotoxicity in CD4 + CD25 − T Cells. *PLoS ONE* 2012;7:e51747.

85. Stocki P, Wang XN, Dickinson AM. Inducible Heat Shock Protein 70 Reduces T Cell Responses and Stimulatory Capacity of Monocyte-derived Dendritic Cells. *J Biol Chem* 2012;287:12387–12394.

86. Hauser GJ, Dayao EK, Wasserloos K, Pitt BR, Wong HR. HSP induction inhibits iNOS mRNA expression and attenuates hypotension in endotoxin-challenged rats. *Am J Physiol - Heart Circ Physiol* 1996;271:H2529–H2535.

87. Chu EK, Ribeiro SP, Slutsky AS. Heat stress increases survival rates in lipopolysaccharide-stimulated rats. *Crit Care Med* 1997;25:1727–1732.

88. Jing L, Wu Q, Wang F. Glutamine induces heat-shock protein and protects against Escherichia coli lipopolysaccharide-induced vascular hyporeactivity in rats. *Crit Care* 2007;11:R34.

89. Wischmeyer PE, Kahana M, Wolfson R, Ren H, Musch MM, Chang EB. Glutamine induces heat shock protein and protects against endotoxin shock in the rat. *J Appl Physiol* 2001;90:2403–2410.

90. McConnell KW, Fox AC, Clark AT, Chang N-YN, Dominguez JA, Farris AB, Buchman TG, Hunt CR, Coopersmith CM. The Role of Heat Shock Protein 70 in Mediating Age-Dependent Mortality in Sepsis. *J Immunol* 2011;186:3718–3725.

91. Hashiguchi N, Ogura H, Tanaka H, Koh T, Nakamori Y, Noborio M, Shiozaki T, Nishino M, Kuwagata Y, Shimazu T, Sugimoto H. Enhanced expression of heat shock proteins in activated polymorphonuclear leukocytes in patients with sepsis. *J Trauma* 2001;51:1104–1109.

92. Zhang R, Wan X, Zhang X, Kang Q, Bian J, Yu G, Wang J, Zhu K. Plasma HSPA12B Is a Potential Predictor for Poor Outcome in Severe Sepsis. *PLoS ONE* 2014;9:.

93. Wheeler DS, Fisher LE, Catravas JD, Jacobs BR, Carcillo JA, Wong HR. Extracellular hsp70 levels in children with septic shock. *Pediatr Crit Care Med J Soc Crit Care Med World Fed Pediatr Intensive Crit Care Soc* 2005;6:308–311.

94. Ramakrishna K, Pugazhendhi S, Kabeerdoss J, Peter JV. Association between heat shock protein 70 gene polymorphisms and clinical outcomes in intensive care unit patients with sepsis. *Indian J Crit Care Med Peer-Rev Off Publ Indian Soc Crit Care Med* 2014;18:205–211.

95. Kiang JG, Bowman PD, Wu BW, Hampton N, Kiang AG, Zhao B, Juang Y-T, Atkins JL, Tsokos GC. Geldanamycin treatment inhibits hemorrhage-induced increases in KLF6 and iNOS expression in unresuscitated mouse organs: role of inducible HSP70. *J Appl Physiol* 2004;97:564–569.

96. Pittet J-F, Lee H, Morabito D, Howard MB, Welch WJ, Mackersie RC. Serum levels of Hsp 72 measured early after trauma correlate with survival. *J Trauma* 2002;52:611–617; discussion 617.

97. Timmermans K, Kox M, Gerretsen J, Peters E, Scheffer GJ, van der Hoeven JG, Pickkers P, Hoedemaekers CW. The Involvement of Danger-Associated Molecular Patterns in the Development of Immunoparalysis in Cardiac Arrest Patients. *Crit Care Med* 2015;43:2332–2338.

98. Donato R. Intracellular and extracellular roles of S100 proteins. *Microsc Res Tech* 2003;60:540–551.

99. Zwadlo G, Brüggen J, Gerhards G, Schlegel R, Sorg C. Two calcium-binding proteins associated with specific stages of myeloid cell differentiation are expressed by subsets of macrophages in inflammatory tissues. *Clin Exp Immunol* 1988;72:510–515.

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

100. Ehrchen JM, Sunderkötter C, Foell D, Vogl T, Roth J. The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. *J Leukoc Biol* 2009;86:557–566.

101. Vogl T, Tenbrock K, Ludwig S, Leukert N, Ehrhardt C, van Zoelen MAD, Nacken W, Foell D, van der Poll T, Sorg C, Roth J. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. *Nat Med* 2007;13:1042–1049.

102. Foell D, Wittkowski H, Kessel C, Lüken A, Weinhage T, Varga G, Vogl T, Wirth T, Viemann D, Björk P, van Zoelen MAD, Gohar F, Srikrishna G, Kraft M, Roth J. Proinflammatory S100A12 can activate human monocytes via Toll-like receptor 4. *Am J Respir Crit Care Med* 2013;187:1324–1334.

103. Raabe A, Grolms C, Sorge O, Zimmermann M, Seifert V. Serum S-100B protein in severe head injury. *Neurosurgery* 1999;45:477–483.

104. Goyal A, Failla MD, Niyonkuru C, Amin K, Fabio A, Berger RP, Wagner AK. S100b as a prognostic biomarker in outcome prediction for patients with severe traumatic brain injury. *J Neurotrauma* 2013;30:946–957.

105. Kecskes Z, Dunster KR, Colditz PB. NSE and S100 after hypoxia in the newborn pig. *Pediatr Res* 2005;58:953–957.

106. Lai PMR, Du R. Association between S100B Levels and Long-Term Outcome after Aneurysmal Subarachnoid Hemorrhage: Systematic Review and Pooled Analysis. *PLoS ONE* 2016;11:e0151853.

107. Achouiti A, Vogl T, Endeman H, Mortensen BL, Laterre P-F, Wittebole X, van Zoelen MAD, Zhang Y, Hoogerwerf JJ, Florquin S, Schultz MJ, Grutters JC, Biesma DH, Roth J, Skaar EP, van 't Veer C, de Vos AF, van der Poll T. Myeloid-related protein-8/14 facilitates bacterial growth during pneumococcal pneumonia. *Thorax* 2014;69:1034–1042.

108. Achouiti A, Vogl T, Van der Meer AJ, Stroo I, Florquin S, de Boer OJ, Roth J, Zeerleder S, van ’t Veer C, de Vos AF, van der Poll T. Myeloid-related protein-14 deficiency promotes inflammation in staphylococcal pneumonia. *Eur Respir J* 2015;46:464–473.

109. Payen D, Lukaszewicz A-C, Belikova I, Faivre V, Gelin C, Russwurm S, Launay J-M, Sevenet N. Gene profiling in human blood leucocytes during recovery from septic shock. *Intensive Care Med* 2008;34:1371–1376.

110. Achouiti A, Föll D, Vogl T, van Till JWO, Laterre P-F, Dugernier T, Wittebole X, Boermeester MA, Roth J, van der Poll T, van Zoelen MAD. S100A12 and soluble receptor for advanced glycation end products levels during human severe sepsis. *Shock Augusta Ga* 2013;40:188–194.

111. Uhle F, Lichtenstern C, Brenner T, Fleming T, Koch C, Hecker A, Heiss C, Nawroth PP, Hofer S, Weigand MA, Weismüller K. Role of the RAGE Axis during the Immune Response after Severe Trauma: A Prospective Pilot Study. *Mediators Inflamm* 2015;2015:.

112. Austermann J, Friesenhagen J, Fassl SK, Ortkras T, Burgmann J, Barczyk-Kahlert K, Faist E, Zedler S, Pirr S, Rohde C, Müller-Tidow C, von Köckritz-Blickwede M, von Kaisenberg CS, Flohé SB, Ulas T, Schultze JL, Roth J, Vogl T, Viemann D. Alarmins MRP8 and MRP14 Induce Stress Tolerance in Phagocytes under Sterile Inflammatory Conditions. *Cell Rep* 2014;9:2112–2123.

113. Wittkowski H, Sturrock A, van Zoelen MAD, Viemann D, van der Poll T, Hoidal JR, Roth J, Foell D. Neutrophil-derived S100A12 in acute lung injury and respiratory distress syndrome: *Crit Care Med* 2007;35:1369–1375.

114. Lorenz E, Muhlebach MS, Tessier PA, Alexis NE, Duncan Hite R, Seeds MC, Peden DB, Meredith W. Different expression ratio of S100A8/A9 and S100A12 in acute and chronic lung diseases. *Respir Med* 2008;102:567–573.

115. Jabaudon M, Blondonnet R, Roszyk L, Pereira B, Guérin R, Perbet S, Cayot S, Bouvier D, Blanchon L, Sapin V, Constantin J-M. Soluble Forms and Ligands of the Receptor for Advanced Glycation End-

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

Products in Patients with Acute Respiratory Distress Syndrome: An Observational Prospective Study. *PLoS ONE* 2015;10:.

116. Shinozaki K, Oda S, Sadahiro T, Nakamura M, Abe R, Nakada T-A, Nomura F, Nakanishi K, Kitamura N, Hirasawa H. Serum S-100B is superior to neuron-specific enolase as an early prognostic biomarker for neurological outcome following cardiopulmonary resuscitation. *Resuscitation* 2009;80:870–875.

117. Calderon LM, Guyette FX, Doshi AA, Callaway CW, Rittenberger JC, Post Cardiac Arrest Service. Combining NSE and S100B with clinical examination findings to predict survival after resuscitation from cardiac arrest. *Resuscitation* 2014;85:1025–1029.

118. Holdenrieder S, Stieber P, Bodenmüller H, Busch M, Von Pawel J, Schalhorn A, Nagel D, Seidel D. Circulating nucleosomes in serum. *Ann N Y Acad Sci* 2001;945:93–102.

119. Huang H, Evankovich J, Yan W, Nace G, Zhang L, Ross M, Liao X, Billiar T, Xu J, Esmon CT, Tsung A. Endogenous histones function as alarmins in sterile inflammatory liver injury through Toll-like receptor 9 in mice. *Hepatology* 2011;54:999–1008.

120. Allam R, Scherbaum CR, Darisipudi MN, Mulay SR, Hägele H, Lichtnekert J, Hagemann JH, Rupanagudi KV, Ryu M, Schwarzenberger C, Hohenstein B, Hugo C, Uhl B, Reichel CA, Krombach F, Monestier M, Liapis H, Moreth K, Schaefer L, Anders H-J. Histones from Dying Renal Cells Aggravate Kidney Injury via TLR2 and TLR4. *J Am Soc Nephrol* 2012;23:1375–1388.

121. Xu J, Zhang X, Monestier M, Esmon NL, Esmon CT. Extracellular Histones Are Mediators of Death through TLR2 and TLR4 in Mouse Fatal Liver Injury. *J Immunol* 2011;187:2626–2631.

122. Huang H, Chen H-W, Evankovich J, Yan W, Rosborough BR, Nace GW, Ding Q, Loughran P, Beer-Stolz D, Billiar TR, Esmon CT, Tsung A. Histones activate the NLRP3 inflammasome in Kupffer cells during sterile inflammatory liver injury. *J Immunol Baltim Md 1950* 2013;191:2665–2679.

123. Abrams ST, Zhang N, Manson J, Liu T, Dart C, Baluwa F, Wang SS, Brohi K, Kipar A, Yu W, Wang G, Toh C-H. Circulating Histones Are Mediators of Trauma-associated Lung Injury. *Am J Respir Crit Care Med* 2013;187:160–169.

124. Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, Taylor FB, Esmon NL, Lupu F, Esmon CT. Extracellular histones are major mediators of death in sepsis. *Nat Med* 2009;15:1318–1321.

125. Alhamdi Y, Abrams ST, Cheng Z, Jing S, Su D, Liu Z, Lane S, Welters I, Wang G, Toh C-H. Circulating Histones Are Major Mediators of Cardiac Injury in Patients With Sepsis. *Crit Care Med* 2015;43:2094–2103.

126. Ekaney ML, Otto GP, Sossdorf M, Sponholz C, Boehringer M, Loesche W, Rittirsch D, Wilharm A, Kurzai O, Bauer M, Claus RA. Impact of plasma histones in human sepsis and their contribution to cellular injury and inflammation. *Crit Care* 2014;18:543.

127. Ingels C, Derese I, Wouters PJ, Van den Berghe G, Vanhorebeek I. Soluble RAGE and the RAGE ligands HMGB1 and S100A12 in critical illness: impact of glycemic control with insulin and relation with clinical outcome. *Shock Augusta Ga* 2015;43:109–116.

128. Hofer S, Brenner T, Bopp C, Steppan J, Lichtenstern C, Weitz J, Bruckner T, Martin E, Hoffmann U, Weigand MA. Cell death serum biomarkers are early predictors for survival in severe septic patients with hepatic dysfunction. *Crit Care* 2009;13:R93.

129. Hotchkiss RS, Nicholson DW. Apoptosis and caspases regulate death and inflammation in sepsis. *Nat Rev Immunol* 2006;6:813–822.

130. Hotchkiss RS, Swanson PE, Freeman BD, Tinsley KW, Cobb JP, Matuschak GM, Buchman TG, Karl IE. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. *Crit Care Med* 1999;27:1230–1251.

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

131. Bonaldi T, Talamo F, Scaffidi P, Ferrera D, Porto A, Bachi A, Rubartelli A, Agresti A, Bianchi ME. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. *EMBO J* 2003;22:5551–5560.

132. Youn JH, Shin J-S. Nucleocytoplasmic Shuttling of HMGB1 Is Regulated by Phosphorylation That Redirects It toward Secretion. *J Immunol* 2006;177:7889–7897.

133. Gardella S, Andrei C, Ferrera D, Lotti LV, Torrisi MR, Bianchi ME, Rubartelli A. The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. *EMBO Rep* 2002;3:995–1001.

134. Clayton A, Turkes A, Navabi H, Mason MD, Tabi Z. Induction of heat shock proteins in B-cell exosomes. *J Cell Sci* 2005;118:3631–3638.

135. Lancaster GI, Febbraio MA. Exosome-dependent Trafficking of HSP70 A NOVEL SECRETORY PATHWAY FOR CELLULAR STRESS PROTEINS. *J Biol Chem* 2005;280:23349–23355.

136. Bausero MA, Gastpar R, Multhoff G, Asea A. Alternative Mechanism by which IFN-γ Enhances Tumor Recognition: Active Release of Heat Shock Protein 72. *J Immunol* 2005;175:2900–2912.

137. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E, Schmid I, Straumann A, Reichenbach J, Gleich GJ, Simon H-U. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. *Nat Med* 2008;14:949–953.

138. Mai SHC, Khan M, Dwivedi DJ, Ross CA, Zhou J, Gould TJ, Gross PL, Weitz JI, Fox-Robichaud AE, Liaw PC, Canadian Critical Care Translational Biology Group. Delayed but not Early Treatment with DNase Reduces Organ Damage and Improves Outcome in a Murine Model of Sepsis. *Shock Augusta Ga* 2015;44:166–172.

139. Simmons JD, Freno DR, Muscat CA, Obiako B, Lee Y-LL, Pastukh VM, Brevard SB, Gillespie MN. Mitochondrial DNA damage associated molecular patterns in ventilator-associated pneumonia: Prevention and reversal by intratracheal DNase I. *J Trauma Acute Care Surg* 2017;82:120–125.

140. Tang D, Kang R, Xiao W, Wang H, Calderwood SK, Xiao X. The Anti-inflammatory Effects of Heat Shock Protein 72 Involve Inhibition of High-Mobility-Group Box 1 Release and Proinflammatory Function in Macrophages. *J Immunol* 2007;179:1236–1244.

141. Tang D, Kang R, Xiao W, Jiang L, Liu M, Shi Y, Wang K, Wang H, Xiao X. Nuclear Heat Shock Protein 72 as a Negative Regulator of Oxidative Stress (Hydrogen Peroxide)-Induced HMGB1 Cytoplasmic Translocation and Release. *J Immunol* 2007;178:7376–7384.

142. Sha T, Sunamoto M, Kitazaki T, Sato J, Ii M, Iizawa Y. Therapeutic effects of TAK-242, a novel selective Toll-like receptor 4 signal transduction inhibitor, in mouse endotoxin shock model. *Eur J Pharmacol* 2007;571:231–239.

143. Rice TW, Wheeler AP, Bernard GR, Vincent J-L, Angus DC, Aikawa N, Demeyer I, Sainati S, Amlot N, Cao C, Ii M, Matsuda H, Mouri K, Cohen J. A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. *Crit Care Med* 2010;38:1685–1694.

144. Mullarkey M, Rose JR, Bristol J, Kawata T, Kimura A, Kobayashi S, Przetak M, Chow J, Gusovsky F, Christ WJ, Rossignol DP. Inhibition of endotoxin response by e5564, a novel Toll-like receptor 4-directed endotoxin antagonist. *J Pharmacol Exp Ther* 2003;304:1093–1102.

145. Lynn M, Rossignol DP, Wheeler JL, Kao RJ, Perdomo CA, Noveck R, Vargas R, D'Angelo T, Gotzkowsky S, McMahon FG. Blocking of responses to endotoxin by E5564 in healthy volunteers with experimental endotoxemia. *J Infect Dis* 2003;187:631–639.

146. Tidswell M, Tillis W, Larosa SP, Lynn M, Wittek AE, Kao R, Wheeler J, Gogate J, Opal SM, Eritoran Sepsis Study Group. Phase 2 trial of eritoran tetrasodium (E5564), a toll-like receptor 4 antagonist, in patients with severe sepsis. *Crit Care Med* 2010;38:72–83.

147. Opal SM, Laterre P-F, Francois B, LaRosa SP, Angus DC, Mira J-P, Wittebole X, Dugernier T, Perrotin D, Tidswell M, Jauregui L, Krell K, Pachl J, Takahashi T, Peckelsen C, Cordasco E, Chang C-S, Oeyen S, Aikawa N, Maruyama T, Schein R, Kalil AC, Van Nuffelen M, Lynn M, Rossignol DP, Gogate J,

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

Roberts MB, Wheeler JL, Vincent J-L, *et al.* Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: the ACCESS randomized trial. *JAMA* 2013;309:1154–1162.

148. Zoelen MAD van, Schouten M, Vos AF de, Florquin S, Meijers JCM, Nawroth PP, Bierhaus A, Poll T van der. The Receptor for Advanced Glycation End Products Impairs Host Defense in Pneumococcal Pneumonia. *J Immunol* 2009;182:4349–4356.

149. Lutterloh EC, Opal SM, Pittman DD, Keith JC, Tan X-Y, Clancy BM, Palmer H, Milarski K, Sun Y, Palardy JE, Parejo NA, Kessimian N. Inhibition of the RAGE products increases survival in experimental models of severe sepsis and systemic infection. *Crit Care Lond Engl* 2007;11:R122.

150. Christaki E, Opal SM, Keith JC, Kessimian N, Palardy JE, Parejo NA, Tan XY, Piche-nicholas N, Tchistiakova L, Vlasuk GP, Shields KM, Feldman JL, Lavallie ER, Arai M, Mounts W, Pittman DD. A Monoclonal Antibody Against Rage Alters Gene Expression and is Protective in Experimental Models of Sepsis and Pneumococcal Pneumonia. *Shock* 2011;35:492–498.

151. Moreno-Gonzalez G, Vandenabeele P, Krysko DV. Necroptosis: A Novel Cell Death Modality and Its Potential Relevance for Critical Care Medicine. *Am J Respir Crit Care Med* 2016;194:415–428.

152. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, NHLBI ARDS Network. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med* 2014;2:611–620.

153. Hu Q, Wood CR, Cimen S, Venkatachalam AB, Alwayn IPJ. Mitochondrial Damage-Associated Molecular Patterns (MTDs) Are Released during Hepatic Ischemia Reperfusion and Induce Inflammatory Responses. *PloS One* 2015;10:e0140105.

154. Szczesny B, Brunyánszki A, Ahmad A, Oláh G, Porter C, Toliver-Kinsky T, Sidossis L, Herndon DN, Szabo C. Time-Dependent and Organ-Specific Changes in Mitochondrial Function, Mitochondrial DNA Integrity, Oxidative Stress and Mononuclear Cell Infiltration in a Mouse Model of Burn Injury. *PLOS ONE* 2015;10:e0143730.

155. Chiu TW, Young R, Chan LYS, Burd A, Lo DYM. Plasma cell-free DNA as an indicator of severity of injury in burn patients. *Clin Chem Lab Med* 2006;44:13–17.

156. Deng Y, Yang Z, Gao Y, Xu H, Zheng B, Jiang M, Xu J, He Z, Wang X. Toll-like receptor 4 mediates acute lung injury induced by high mobility group box-1. *PloS One* 2013;8:e64375.

157. Kim JY, Park JS, Strassheim D, Douglas I, Diaz del Valle F, Asehnoune K, Mitra S, Kwak SH, Yamada S, Maruyama I, Ishizaka A, Abraham E. HMGB1 contributes to the development of acute lung injury after hemorrhage. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L958-965.

158. Xu M, Zhou G-M, Wang L-H, Zhu L, Liu J-M, Wang X-D, Li H-T, Chen L. Inhibiting High-Mobility Group Box 1 (HMGB1) Attenuates Inflammatory Cytokine Expression and Neurological Deficit in Ischemic Brain Injury Following Cardiac Arrest in Rats. *Inflammation* 2016;39:1594–1602.

159. Huang L-F, Yao Y-M, Zhang L-T, Dong N, Yu Y, Sheng Z-Y. The effect of high-mobility group box 1 protein on activity of regulatory T cells after thermal injury in rats. *Shock Augusta Ga* 2009;31:322–329.

160. Oda Y, Tsuruta R, Fujita M, Kaneda K, Kawamura Y, Izumi T, Kasaoka S, Maruyama I, Maekawa T. Prediction of the neurological outcome with intrathecal high mobility group box 1 and S100B in cardiac arrest victims: a pilot study. *Resuscitation* 2012;83:1006–1012.

161. Lantos J, Földi V, Roth E, Wéber G, Bogár L, Csontos C. Burn trauma induces early HMGB1 release in patients: its correlation with cytokines. *Shock Augusta Ga* 2010;33:562–567.

162. Vabulas RM, Ahmad-Nejad P, Costa C da, Miethke T, Kirschning CJ, Häcker H, Wagner H. Endocytosed HSP60s Use Toll-like Receptor 2 (TLR2) and TLR4 to Activate the Toll/Interleukin-1 Receptor Signaling Pathway in Innate Immune Cells. *J Biol Chem* 2001;276:31332–31339.

163. Gong J, Zhu B, Murshid A, Adachi H, Song B, Lee A, Liu C, Calderwood SK. T cell activation by heat shock protein 70 vaccine requires TLR signaling and scavenger receptor expressed by endothelial cells-1. *J Immunol Baltim Md 1950* 2009;183:3092–3098.

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

164. Pawaria S, Binder RJ. CD91-dependent programming of T-helper cell responses following heat shock protein immunization. *Nat Commun* 2011;2:521.

165. Asea A, Kraeft S-K, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK. HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med* 2000;6:435–442.

166. Baker TA, Romero J, Bach HH, Strom JA, Gamelli RL, Majetschak M. Systemic release of cytokines and heat shock proteins in porcine models of polytrauma and hemorrhage*. *Crit Care Med* 2012;40:876–885.

167. Villar J, Cabrera N, Casula M, Flores C, Valladares F, Muros M, Blanch L, Slutsky AS, Kacmarek RM. Mechanical ventilation modulates Toll-like receptor signaling pathway in a sepsis-induced lung injury model. *Intensive Care Med* 2010;36:1049–1057.

168. Ganter MT. Extracellular heat shock protein 72 is a marker of the stress protein response in acute lung injury. *AJP Lung Cell Mol Physiol* 2006;291:L354–L361.

169. Hiroshima Y, Hsu K, Tedla N, Wong SW, Chow S, Kawaguchi N, Geczy CL. S100A8/A9 and S100A9 reduce acute lung injury. *Immunol Cell Biol* 2017;95:461–472.

170. Jeppesen AN, Hvas A-M, Grejs AM, Duez CHV, Sorensen BS, Kirkegaard H. Post-cardiac arrest level of free-plasma DNA and DNA-histone complexes. *Acta Anaesthesiol Scand* 2017;61:523–531.

DAMP	Receptor	Non-human studies	Human studies
Mitochondrial DNA	TLR9 (39), 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), 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), 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), RAGE (41)	Sepsis (107, 108) Trauma (103, 104) ALI (169)	Sepsis (109, 110) Trauma (111, 112) ALI (113–115)

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

			Cardiac Arrest (18, 116, 117)
Histones	TLR2 (120, 121), TLR4 (120, 121), TLR9(119) NLPR3 (122)	Sepsis (124) Trauma (123) ALI (123)	Sepsis (125, 126) Trauma (123) ALI (123) Cardiac Arrest (170)

Table 1:

Table 1: Five commonly reported DAMPs, their reported receptors, studies preclinical models of critical illness, and studies of human cohorts of critical illness. **TLR** = toll like receptor, **ALI** = acute lung injury, **RAGE** = receptor for advanced glycation end-products, **VILI** = ventilator induced lung injury, **SREC-1** = scavenger receptor expressed by endothelial cells I

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.

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 Toll-like Receptors (TLRs) and Pathogen-Recognition Receptors (PRRs) leading to activation of the NF-kB. NF-kB translocates to the nucleus and promotes expression of pro-IL-18, pro-IL1β and NLRP3. NLRP3 is de-ubiquinated and combines with apoptosis-associated speck-like protein (ASC) and pro-caspase-1 to form the NLRP3 inflammasome. The

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

activated NLRP3 inflammasome converts pro-caspase-1 to caspase-1. Caspase-1 subsequently then catalyzes the production of mature IL-18 and IL-1β.

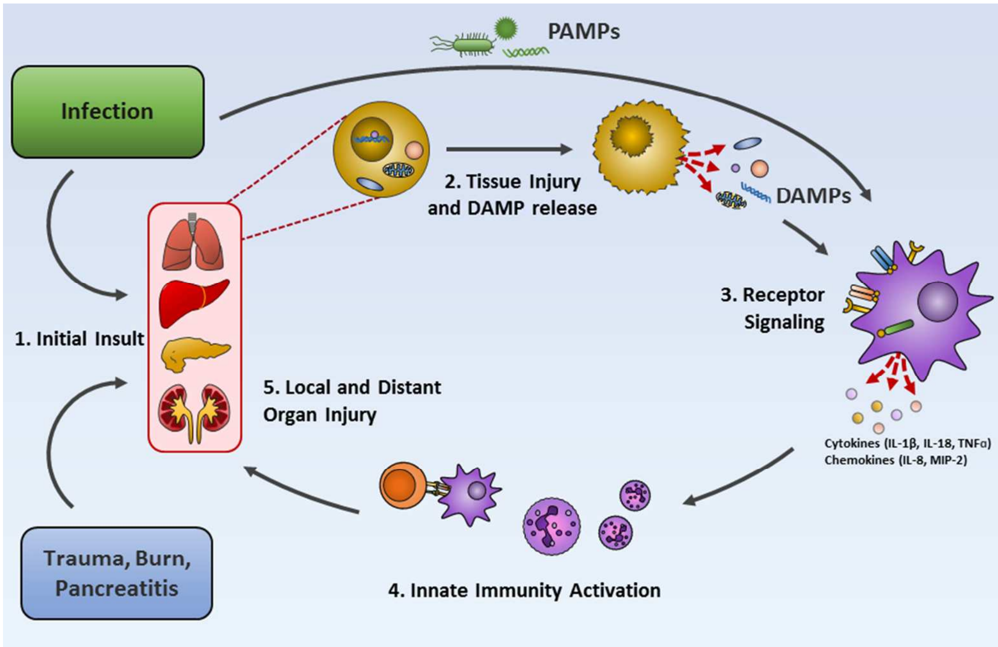

Figure 1. Proposed schema for the pathogenesis of damage-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP) mediated local and distal organ injury during critical illness. 1) Infection or sterile organ injury such as trauma, burns, or pancreatitis leads to direct initial 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.

AJRCCM Articles in Press. Published on 04-October-2017 as 10.1164/rccm.201612-2460PP

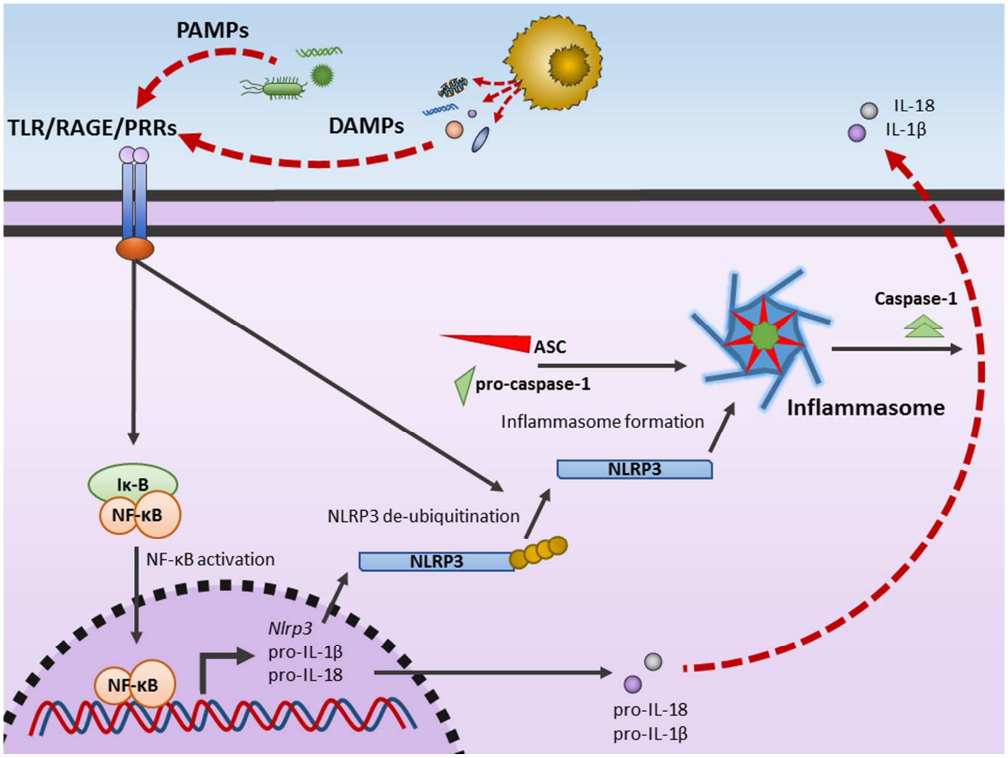

Figure 2. Schema of damage-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP) mediated activation of innate immunity and inflammation. PAMPs and DAMPs released from infection and tissue injury, respectively, bind cell surface Toll-like Receptors (TLRs) and Pathogen-Recognition Receptors (PRRs) leading to activation of the NF-kB. NF-kB translocates to the nucleus and promotes expression of pro-IL-18, pro-IL1β and NLRP3. NLRP3 is de-ubiquinated 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.