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Danger Signals in the ICU

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Abstract

Objective—Sterile and infectious critical illnesses often result in vasoplegic shock and a robust systemic inflammatory response that are similar in presentation. The innate immune system is at the center of the response to both infectious and traumatic insults. Damage associated molecular patterns (DAMPs) are small molecules that are released from stressed or dying cells. DAMPs activate pattern recognition receptors and coordinate the leading edge of the innate immune response. This review introduces the concept of DAMPs and how they activate a systemic inflammatory response, specifically in trauma, neurologic injury and infection. It also explores how, when carried to extremes, DAMPs may even perpetuate multisystem organ failure.

Data Sources—Basic and clinical studies were obtained from a PubMed search through August 2017.

Study Selection—Articles considered include original articles, review articles and conference proceedings.

Data Extraction—An analysis of scientific, peer-reviewed data was performed. High quality pre-clinical and clinical studies adjudicated by the authors were included and summarized.

Data Synthesis—Pattern recognition receptors respond to DAMPs and then activate inflammatory pathways. DAMPs have been linked to the recruitment of sentinel leukocytes and the initiation of the inflammatory cascade. DAMPs have been linked to many conditions in critical care illnesses. Preclinical models have added insight into how they may mediate distant organ dysfunction.

Conclusions—DAMP activation and release is important research for intensive care practitioners. It will add to our understanding of the phase and state of the innate immune response to an insult. Early work is encouraging. However, only with improved understanding of DAMP

activation and function, we can perhaps hope to target DAMPS as diagnostic and/or therapeutic modalities in the future.

Keywords

Damage-associated molecular patterns; DAMPs; sterile systemic inflammatory response; Pathogen associated molecular patterns; PAMPs; Innate Immune system

Introduction

“I think it likely that many of our diseases work in this way. Sometimes, the mechanisms used for overkill are immunologic, but often, they are more primitive kinds of memory. We tear ourselves to pieces because of symbols, and we are more vulnerable to this than to any host of predators. We are, in effect, at the mercy of our own Pentagons, most of the time.”

Lewis Thomas MD, *Germes. The Lives of a Cell* 1974(1)

The innate immune system is central to the appropriate control of localized infections and the coordination in wound healing.(2) This system is designed to prevent take over from foreign genomes and to remove damaged cells in order to promote wound healing.(3, 4) The ability to kill and remodel are integral to this process.(5) Due to the evolutionary conservation of cellular structures, removal of invading threats and responding to traumatic insults can be accompanied by collateral damage.(4) Carried to extremes, tissue destruction continues beyond homeostatic blockades and results in disseminated inflammation and multi-organ dysfunction.(6) Despite decades of research dedicated to late effectors of inflammation in critical care illness, attempts to modulate the host response in trauma, cardiac arrest and syndromes such as sepsis and acute respiratory distress have been met with failure.(7-11) This suggests an incomplete understanding of the innate immune response in these conditions.

Recent progress in research is shedding light on these processes. When faced by a threat by either tissue destruction or pathogen, any cell of the body is capable of sounding an alarm. (2) Stressed or dying cells release molecules known damage associated molecular patterns (DAMPs) that reside in all cells and tissues.(12, 13) DAMPs are integral danger signals to surrounding tissues and the innate immune system. Severe stressors and injuries mobilize these DAMPS from their normal location and cause them to activate innate immunity. Through this mechanism, injured cells and tissues trigger an immunologic alarm and provoke inflammation.(14, 15) DAMPS, therefore, play a key role in control of pathogens, removal of necrotic debris and repair of injured tissues.(16) However, DAMPS may also unleash a dysregulated or excessive immune response that generates both the systemic inflammatory response syndrome and multiple organ dysfunction.(6) Excellent, broad, reviews concerning the role of DAMPs in general critical illness have recently been published elsewhere.(17, 18)

This review will introduce the concept of DAMPs in critical illness. It will review how DAMPs engage the innate immune system and activate inflammation. We will then explore how innate immune based inflammation can lead to progressive non-apoptotic cell death and

multi-organ dysfunction. Lastly, we will summarize the evidence in human studies of trauma, neurologic injury and infection linking DAMPs to outcomes and explore the potential for modulation of DAMP signaling in critical illness. This review offers a needed synthesis of the translational interplay of DAMPs and the innate immune response.

What is a DAMP

Understanding of the innate immune system's role in acute illness has undergone a massive upheaval in the past 25 years. Beginning with the immunologic theory of the "Infectious non-self" by Janeway in the late 1980's,(19, 20) our concept of the first response in acute illness have grown. Breach of an epithelial lining by a pathogen is followed by the innate immune system quickly identifying these threats through the recognition of key components of the invader.(21) Peptidoglycans, double stranded pathogen DNA, and the canonical endotoxin are able to bind with antigen presenting cells to initiate an inflammatory response. This class of molecules is known as pathogen-associated molecular patterns (PAMPs).(19) The cellular receptors for these molecules are evolutionarily ancient, preserved, moieties defined as pattern recognition receptors (PRR). PRR broadly bind multiple PAMPs from a multitude of bacteria, viruses and other pathogens. They are typified by the Toll-like receptors (TLR), receptors for advanced glycation end products (RAGE) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLR), all of which, upon activation, transduce nuclear transcription.(22) These ligand receptor interactions are linked to the activation of inflammatory cytokines and the display of antigen to the adaptive immune system.(23)

Unfortunately, infection is the not the only cause of critical illness, and organ failure can persist beyond the control of the initial pathogen load. In the mid-1990s, a complimentary model was developed which moved beyond PAMPs to the revolutionary idea that the innate immune system responds not only to the "infectious non-self" but also to any sign of significant destruction or immunogenic cell death.(24-26) Experimental evidence for these concepts emerged over the past 15 years with documentation that host intra and extracellular material can stimulate the aforementioned PRRs.(22) This group of material has been termed DAMPs.(12, 13)

Multiple candidate DAMPS have been proposed over the past decade including mitochondrial and nuclear nucleic acids, histone associated proteins, ATP, heat shock proteins and extracellular matrix (ECM) components such as proteoglycans and heparin sulfate.(27, 28) Characteristics of candidate DAMPs include receptor agonism at physiologic doses and the release from cells under conditions of stress and death.(29) Intracellular DAMPs are composed of molecules that are normally integral to cellular function in non-stressed conditions. They do not engage the immune system in these conditions, distinguishing them from inflammatory cytokines. DAMPs activate PRR's, and the subsequent immune response, when exposed to the extracellular environment.(14) Extracellular DAMPs are able to bind PRR following tissue destruction in their soluble form.(30) Table 1 lists candidate DAMPs their known receptors with known down-stream mediators.

DAMPs and the activation of the innate immune system

Necrotic cell death, from trauma or pathogen toxin, leads to the release of intracellular DAMPs.(14) Early chemotaxis of early innate immune cells, typified by neutrophils, to site of tissue damage is due to transcription-independent DAMP signaling.(5, 31) Zebrafish are excellent models of neutrophil migration.(32) In the Zebrafish, early signaling is accomplished through the enzymatic creation of a H₂O₂ gradient by dual oxidase 1 in response to increased extracellular concentrations of ATP, a putative DAMP.(33) Subsequently, the release of N-formyl peptides is associated with the release of other neutrophil chemotactic factors.(28) Sustained neutrophil recruitment requires the release and activation of chemokines including members of the CXCL8 family and lipid mediators derived from arachadonic acid.(2)

Once at the site of injury or infection, neutrophils, resident macrophages, and dendritic cells coordinate the inflammatory response through DAMP-PRR signaling and subsequent inflammasome activation. Inflammasomes, typified by NLRP3 subtype, are multimeric protein complexes that assemble in the cytosol and are implicated in the activation of the innate immune system.(34) PRR binding by various DAMPs leads to signal transduction and nuclear factor- κ B (NF- κ B) induced gene expression of NLRP3 related proteins. The subsequent formation of the NLRP3 inflammasome is dependent on the release of mitochondrial reactive oxygen species and mitochondrial DNA (mtDNA). ATP then activates the NLRP3 inflammasome through G-protein receptor pathways and the generation of potassium efflux from the cytosol. Once armed, inflammasomes in turn activate caspase 1, which convert the potent inflammatory mediators interleukin-pro-1beta (IL-1 β) and pro-interleukin-18 (IL-18) into their biologically active form.(34, 36) IL-1 β has a multitude of effects, mainly through the induction of gene expression; it activates inducible nitric oxide synthase (iNOS), and the synthesis of cyclooxygenase type 2 and phospholipase A. With these enzymes stimulated, there is local pyrexia, hyperalgesia and vasodilation. IL-1 β further stimulates the production of other proinflammatory cytokines such as IL-6.(37) In addition, local mesenchymal cells and endothelial cells increase the expression of adhesion molecules that promote leukocyte migration and activation. In addition to inflammasome activationDAMP- PRR binding is consistently implicated in the transduction of the NF- κ B gene inductionpathway leading to additional proinflammatory cytokine release such as TNF- α and IL-6.(38)

Local inflammation is integral to the control of infections through the activation of professional leukocytes for phagocytosis, release of reactive oxygen species, and other effector molecules for killing. This response is coordinates early tissue remodeling, neovascularization, and activation of pro-growth and repair pathways.(39) DAMPs cause dendritic cell maturation, which in turn stimulates the adaptive immune system and lasting immunologic memory.(40) The magnitude of the local effect is usually closely correlated with the size of the insult. These early molecular signals are responsible for the coordination of more advanced wound healing and organ recovery.(41, 42)

Excessive innate immune activation and the loss of homeostasis

With large amounts of tissue destruction, a significant pathogen load, or a particularly virulent organism the inflammatory response can become dysregulated. Local release of cytokines and the activation of inflammation can be excessive in response to large stimuli. In these settings, cytokine effects are carried beyond the immediate paracrine environment and into the systemic circulation.⁽¹¹⁾ The cytokines IL-1 β , IL-6, TNF α , and others, activate the endothelium, and cause complement fixation.⁽⁴³⁾ In addition, nonspecific innate immune cells such as neutrophils and macrophages release reactive oxygen species and toxic granular components, leading to necrosis of local tissues.⁽⁵⁾ This tissue destruction releases further DAMPs, which in turn lead to ongoing activation of the immune response. Figure 1 displays a conceptual model of the local response to tissue injury with systemic activation of the innate immune system and distal organ dysfunction through the cycle of DAMP activation.

A link between the mechanistic activation of the innate immune system and the perpetuation of organ dysfunction is the discovery of non-apoptotic programmed cell death.⁽⁴⁴⁾ With exuberant inflammation, tissue cells undergo non-apoptotic programmed cell death typified by necroptosis and pyroptosis.^(45, 46) Activated by alternative cell death pathways tied to DAMP signaling, these cell death mechanisms lead to the further release of DAMPs perpetuating the systemic response.^(45, 47) Importantly, apoptotic cell death is not linked with the release of DAMPs. Neutrophils and macrophages release DNA leading to the creation of extracellular traps (ET) which impede flow through the circulation and can engulf and destroy bacteria and the body's own cells a process termed ETosis.⁽⁴⁸⁾ Immunologically active cell death also leads to potent cytokine release distant to local injury and leads to organ dysfunction and the perpetuation of ongoing cytokine release.^(46, 49)

Clinical Evidence in Critical Illness

Traumatic Injury

Clinical evidence of these phenomena is available from many different sources. It is well known that those with severe trauma that survive the initial insult are susceptible to further organ dysfunction and later infection.^(43, 50, 51) There has been work in various traumatic/surgical injury states connecting serum levels of DAMPs to the severity of initial insult and subsequent risk of complications.

Mitochondrial DNA (MtDNA) is perhaps the most attractive DAMP in trauma, due to the similarity to bacterial DNA, and the importance of mitochondria in controlling cellular response to stress. In a seminal report, Hauser, Zhang, and colleagues presented the first compelling data from 15 severely injured trauma patients (Injury Severity Score > 25)⁽⁵²⁾ where mtDNA levels were many fold higher than controls and were locally increased in femur surgery specimens.⁽²⁸⁾ An additional study by Gu et al demonstrated that mtDNA levels in trauma patients admitted to the ICU were elevated compared to healthy controls and correlated with severity of the inflammatory response and the development of multisystem organ failure.⁽⁵³⁾

Cell free nuclear DNA has also been evaluated as a general marker of cell injury in trauma. In a small cohort of blunt trauma patients plasma levels of nuclear DNA measured within the first hour of arrival to the ED correlated with the upfront injury severity score ($r=0.756$ p -value= <0.001) and had excellent performance predicting lung injury and death compared to the injury score alone.(54)

In addition to nucleic acids, high-mobility group box-1 (**HMGB-1**), an evolutionarily preserved histone-associated protein and one of the earliest discovered DAMPs,(55, 56) has also been studied in sterile traumatic conditions. Important human data from a cohort of 168 patients admitted to a large academic medical center links HMGB-1 with the severity of traumatic injury.(57) In this population there was a strong correlation between upfront HMGB-1 and injury severity score and base deficit. HMGB-1 was increased in blunt, as opposed to penetrating, trauma and importantly, early levels were correlated with the later development of acute lung injury, renal failure, and mortality.(57) In another smaller cohort study, plasma HMGB-1 were evaluated at several time points following admission to the SICU.(58) This study demonstrated elevated levels compared to healthy controls and peaked at 6 hours post injury. However, the levels in this study did not correlate with the injury severity score and organ dysfunction, but did predict a prolonged ICU course.(58) One further study, examining only patients with blunt trauma in the SICU, again demonstrated that upfront and serial HMGB-1 levels correlated with the later development of multisystem organ failure, mortality, and in this cohort secondary sepsis.(59)

Heat shock proteins (HSP) are ubiquitous chaperone proteins conserved across species and are involved in the control of protein folding. There are 13 known human HSP70 subtypes that in situations of stress can exhibit pro and anti-inflammatory properties.(60) HSP72 release and increased levels compared to healthy controls, has been associated with a controlled immune response and improved survival in several trauma cohorts.(61, 62) These analyses hold true even after correcting for underlying severity of illness.(61) Conversely, increased HSP60 in subjects with high baseline severity of illness has been associated with the development of clinical ARDS.(63)

Central Nervous System Injury

Neurological emergencies entail a primary injury, followed by secondary injury believed to be orchestrated by inflammation.(64) Emerging evidence has incriminated this secondary injury as an independent predictor of poor outcomes.(65, 66) The interaction between CNS (central nervous system) and systemic inflammation is complex and remains poorly understood. Current evidence suggest it may be caused by the release of DAMPs in the CNS, which then leak into the peripheral circulation due to disruptions of the blood-brain barrier, leading to the activation of the pro-inflammatory cytokines and chemokines.(67)

HMGB-1 is released from dying neurons in proportions related to the extent of neurological injury.(68) HMGB-1 was shown to correlate with poor functional outcome after ischemic stroke, with a sensitivity comparable to the conventional National Institutes of Health Stroke Scale (NIHSS) (69). Additionally, when used in combination with the NIHSS, HMGB-1 significantly increased the area under the curve for predicting disability compared to the NIHSS alone.(69) Similarly, HMGB-1 has also shown promise in acute subarachnoid

hemorrhage, where it is complementary to clinical severity assessments to predict disability at the end of one year.(70) In patients with high-grade subarachnoid hemorrhage, HMGB-1 levels also appear to independently predict onset of acute hydrocephalus.(71)

Studies correlating other DAMPs with neurologic injury are less mature. **MtDNA** is elevated in the acute phase of ischemic stroke, but its association with outcome is not established.(72) However, in patients with acute bacterial meningitis, increased mtDNA levels were correlated with major disability or death at 90 days.(73) Other DAMPs studied in acute brain injury include heat shock proteins, and S100 proteins.(74)

Infection

In conditions of severe infection, various DAMPs have been associated with poor outcomes and increased baseline severity of illness. The initial pathogen load and PAMP-mediated activation of the immune response is thought to be augmented by subsequent necrotic and programmed cell death with release of endogenous DAMPs, which may perpetuate inflammation despite control of the original infection. In a large study of two medical ICU cohorts, elevated early **mtDNA** levels were noted to be significantly associated with mortality, odds ratio 7.5 CI 3.6–15.8, $p=1\times10^{-7}$ and 8.4 CI 2.9–24.2, $p=1\times10^{-5}$ respectively.(75) In this study, **MtDNA** augments the clinical prediction of inpatient mortality using a model based on the APACHEII score.(75) **Plasma cell free DNA** is associated with septic shock when compared to normal controls in another ICU cohort.(76) **HMGB-1** is also associated with higher mortality in patients with septic shock.(77) Circulating histones are another well studied DAMP and in a small comparative study, elevated levels compared to controls in a mixed ICU population were associated with sepsis and multiple organ failure and poor outcomes.(78)

Other Critical Illnesses

With numerous additional models of individual organ injury DAMPs are usually elevated, including drug induced pancreatitis(79) and liver injury.(80) DAMPs are also elevated in diffuse immune activation states such as graft versus host disease.(81) Other forms of acute illness such as pulmonary embolism and acute myocardial infarction and heart failure are also associated with increased levels of DAMPs which are associated with poor outcomes.(82, 83)

In addition, with improving early intensive care it is clear that those living through the brisk systemic immune response are now at risk for a compensatory anti-inflammatory response that follows a large systemic insult associated with inflammation and catabolism.(84) DAMP release and immune reactivation are linked with this concept although our understanding of it remains in its early stages.(62)

Summary

DAMPs are integral in the early coordination of the innate immune system in a variety of acute illnesses. Specifically, they convey danger to other cells, trigger inflammation, and activate the innate immune system to stop further damage. DAMPs also organize tissue

healing and participate in communication to inform the appropriate adaptive immune response.(14, 40)

The knowledge the immune system responds similarly to traumatic, ischemic and pathogen associated threats offers great insight into the clinical characteristics of the patients seen under our care. This is indeed an attractive theory that both share the same pathway. It is also clear that controlled release and activation of the immune system is needed to appropriately respond to danger. These responses are designed to clear damaged cells and to stop potential invasion of pathogens through lost barrier integrity.(5, 14, 39) The overwhelming, exuberant, and dysregulated response could be modified for clinical benefit.(85) However, even with our rudimentary understanding of DAMP release and immune activation the task is daunting. Multiple DAMPs are released with cell death and in turn activate many receptors with redundant pathways leading to immune coordination. Several trials that have blocked the best studied PAMP/TLR interaction (lipopolysaccharide and TLR-4) have been unsuccessful in modifying further cytokine release or improving patient centered outcomes. (86, 87) Under normal conditions, most molecules that can act as DAMPs exist in the organism and are essential for its continued survival. Therefore, a key aspect of futures studies will be targeting these molecules specifically in their role as DAMPs but without hindering their normal role in the body. In the near term it is not clear who could benefit from modulation of these responses in the acute setting. Indiscriminant blocking of the immune response with our current understanding of the basic science is unlikely to help patients.(88, 89)

Specifically, questions that must be answered in order to achieve the ability to modulate DAMP response include the following. How do DAMPs interact with one another? There are multiple classes released with destruction of a single cell. Are the effects of multiple DAMPs interacting with multiple PRRs complementary or redundant? Is there a hierarchical nature of DAMP-PRR interactions? Additionally, what are the effects of DAMP-PRR interactions in non-immune cells? How do PAMPs and DAMPs interact in the setting of infection, is there augmentation or does sequential activation of the same PRR lower responsiveness?

Conclusions

DAMPs are a representation of the conservative nature of evolution and functional biology. The utility of these hard working cellular machinery components in response to stress to orchestrate the immune response to injury is remarkable. We must further understand DAMP receptor interactions in preclinical models. Additionally, the need is great to understand, on a more complete scale, the type of interaction that these molecules have with one another and the innate immune system. Large clinical observational studies measuring multiple DAMPs, at multiple time points in critical illness will be integral to building this knowledge. DAMPs may offer insight into the state and phase of the immune response and offer tantalizing potential for therapeutics. With improved understanding of the signaling pathways of DAMP activation and importantly of the precise function of DAMPs, we are challenged to perhaps target DAMPS as diagnostic and/or therapeutic modalities in the future.

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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. Infection or sterile organ injury such as trauma, or pancreatitis leads to direct initial insult. Infection and local tissue injury releases PAMPs and DAMPs. PAMPs and DAMPs bind Toll-Like Receptors and Pathogen Recognition Receptors with subsequent cytokine and chemokine release. Activation of the innate immune system with neutrophil recruitment and T-cell activation which leads to resultant local and distant organ injury.

Table 1**DAMP Receptor Interactions**

Cellular Compartment	DAMP	Receptors	Downstream Mediators	Reference
Mitochondria	Mitochondrial DNA	TLR9, AIM2	NF- κ B, TNF α , IL-6, IFN α , IL-1 β , IL-18,	(25)
	ATP	P2X7, P2Y2	Ca ²⁺ , K ⁺ efflux, NLRP3 activation	(86)
Nucleus	Nuclear DNA	TLR9	NF- κ B, TNF α , IL-6	(87, 88)
	HMGB1	TLR2, TLR4, TLR9, RAGE	NF- κ B κ , TNF α , IL-6, IFN α	(51)
	Histones	TLR2, TLR4, TLR9, NLRP3	NF- κ B, IL-1 β , IL-18, TNF α , IL-6, IFN α	(89)
Cytoplasm	Heat Shock Proteins	TLR2, TLR4	NF- κ B, TNF α , IL-6, IFN α	(90)
	S100 Proteins	TLR4, RAGE	NF- κ B, TNF α , IL-6, IFN α	(91)
	Uric acid	NLRP3	IL-1 β , IL-18, IL-6, IFN α	(92, 93)
Extracellular Matrix	Proteoglycans	TLR2, TLR4, NLRP3	NF- κ B, IL-1 β , IL-18, TNF α , IL-6, IFN α	(94, 95)
	Heparan Sulfate	TLR4	NF- κ B, TNF α , IL-6, IFN α	(77, 96)
	Fibronectin	TLR4	NF- κ B, TNF α , IL-6, IFN α	(97)
	Fibrinogen	TLR4	NF- κ B, TNF α , IL-6, IFN α	(98)

DAMPs, their reported receptors, purported down-stream mediators. **TLR** = toll like receptor, **RAGE** = receptor for advanced glycation end-products, **NLRP3** = NACHT LRR PYD 3, AIM 2 = Absent in Melanoma