Damage- and Pathogen-Associated Molecular Patterns and Alarmins: Keys to Sepsis?

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Damage-associated molecular patterns ·
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Abstract

The concept that sepsis is the result of an uncontrolled inflammatory response of the host's innate immune system towards invading pathogens has recently been challenged. Evidence is accumulating that, in addition, host-derived alarm molecules are released during sepsis- and trauma-associated cell death, thus triggering the host's immune response. The identification and characterization of exogenous as well as endogenous danger molecules allowed significant advances in our understanding of the pathophysiology of sepsis and may provide potential targets for therapeutic interventions.

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New Definitions of Old Players: Damage- and Pathogen-Associated Molecular Patterns and Alarmins

In 1891, the Russian scientist Elie Metchnikoff was the first to discover phagocytosis, a cell-mediated immune response against foreign microorganisms [1]. More than

a century later, Charles Janeway proposed that the innate immune system uses evolutionarily ancient pattern recognition receptors such as Toll-like receptors (TLRs) and mannose receptors for the detection and identification of invading pathogens and their pathogen-associated molecular patterns (PAMPs) [2, 3]. However, Janeway's model did not explain the immune response against 'self tissue' transplants, tumors, ischemia-related injuries or autoimmune diseases. In 2004, Seong and Matzinger [4] proposed that the immune system not only recognizes exogenous pathogens but also senses and responds to endogenous alarm signals released through cellular stress, trauma-induced tissue damage or nonphysiological cell death such as necrosis. This danger model is based on the fact that life evolved in water, which drives the hydrophobic portions (hyppos) of molecules to separate from water and aggregate with each other. Under physiological conditions, hyppos are hidden within lipid membranes of cells or other intracellular compartments. Although 'self' molecules are potentially immunoreactive, they are shielded within the cell, so that they are not sensed by the host immune system. However, the sudden exposure of intracellular molecules or their derivatives on the outer leaflet acts as a sign of a potential danger of cellular damage [4]. Therefore, the recognition patterns of pathogens might be similar to those of released endogenous molecular structures, recognized by an evolutionarily ancient 'alarm system' for *danger*-associated molecular patterns.

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Although the detection of endogenous and exogenous alarm signals is possibly accomplished by the same early recognition systems, the source of danger remains different, causing some confusion and controversy about the terms damage-associated molecular patterns (DAMPs), PAMPs and alarmins. In regard to their discovery, the immunogenic foreign pathogen motifs were considered distinct from host-encoded molecules and therefore referred to as PAMPs, i.e. *pathogen*- (from Greek 'pathos' and 'genēs': 'producer of disease') associated molecular patterns [3]. Analogous, endogenous products released from dying or damaged cells were called DAMPs, i.e. *damage*- (from Latin 'damnum': 'loss, hurt') associated molecular patterns [4]. Based on the at least in part common recognition system and the similar inflammatory response, some authors use the term DAMPs as a unifying danger concept, integrating PAMPs with endogenous alarm signals. In this regard, the term alarmin (Italian 'all'arme': 'to arms') has been proposed to categorize endogenous DAMPs as separate from 'nonself' pathogen-derived molecules [5]. However, pathogen-associated exogenous derivatives do not necessarily involve tissue damage, whereupon 'danger associated' would better reflect the common concept than 'damage associated'. We therefore use the term alarmin to differentiate the endogenous danger signals, which sense tissue and cell damage, from exogenous invading pathogens, unifying both in the large family of *danger*- (from French 'dangier': 'power to harm') associated molecular patterns.

Sepsis

In the USA, sepsis is the second leading cause of death within noncoronary intensive care units, with increased mortality rates of between 20% for sepsis and 60% for septic shock [6]. Although the term 'sepsis' has been used for more than 2,700 years, the clinical definitions of sepsis were rather confusing and only inadequate. In 1991, standardized definitions for the various stages of sepsis were proposed to facilitate clinical assessment and ongoing research in this field [7]. Sepsis was defined as a clinical syndrome characterized by systemic inflammation (systemic inflammatory response syndrome, SIRS) with detection of bacteria. SIRS is manifested by two or more of the following conditions: hyperthermia ($>38.3^{\circ}\text{C}$) or hypothermia ($<35.5^{\circ}\text{C}$), tachycardia (>90 beats/min), tachypnea (>20 breaths/min) and white blood count $>12,000/\text{mm}^3$ or $<4,000/\text{mm}^3$ or with $>10\%$ immature

neutrophils. Later concepts such as the predisposition, insult/infection, response and organ dysfunction model also include predisposing factors and premorbid conditions [8].

The underlying pathophysiology of both SIRS and sepsis is rooted in the body's innate immune system. Unlike the adaptive immune system, innate immunity is ready to immediately respond once immune cells encounter molecular patterns typical of invading pathogens or endogenous alarm signals from damaged cells [2] (fig. 1). The recognition of these DAMPs by recognition receptors of the innate immune system (danger sensing) results in an early activation of various serine protease systems such as the coagulation and complement cascades [9]. The subsequent activation of the 'first line of cellular defense' leads to an excessive activation of various intracellular signaling pathways and the release of cytokines and chemokines, accompanied by a complex neuroendocrine reaction [10]. In particular, cytokines and the transmigration of neutrophils and monocytes may result in endothelial dysfunction, causing vasodilatation and increased capillary permeability. The resulting leakage syndrome is clinically associated with hypotension, hemoconcentration, macromolecular extravasation and global edema [11, 12] (fig. 2). The dysfunctional endothelial barriers (blood-brain, blood-lung, blood-bowel and blood-urine barriers) enable further invasion by microorganisms and their products, thus disturbing the body's regulatory mechanisms on a molecular, cellular, tissue, organ and multiorgan level (fig. 2).

Recent findings suggest that the patient's response to sepsis involves both pro- and anti-inflammatory processes [13]. In this regard, sepsis results in a cascade of immune-modulatory events dominated by a hyperinflammatory state and the production of proinflammatory cytokines, including interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α [14]. Almost simultaneously with the initial hyperinflammatory response, generation of anti-inflammatory cytokines occurs, including IL-10, followed by apoptosis of immune effector cells [14]. IL-10 has been shown to predict poor outcome in septic patients, and modulation of IL-10 in animal models of sepsis was found to improve survival.

Enhanced levels of various inflammatory markers such as IL-6, TNF- α or C-reactive protein have been identified in patients with sepsis as correlating with the clinical outcome [15]. However, none of these factors was an ideal sepsis marker as they were all indicators of inflammation rather than infection, and none was proven to be 100% specific for sepsis. Based on the danger mod-

Fig. 1. Host response to molecular danger. Exogenous (pathogens) and endogenous (alarmins) danger-associated molecular patterns are recognized by the host's innate immune system, resulting in a systemic immune response, clinically manifested as sepsis.

el, the search for adequate surrogate sepsis markers was expanded to endogenous biomarkers that are released into the circulation as a sign of danger in the case of sepsis.

Exogenous PAMPs in Sepsis

PAMPs are molecular motifs consistently found on pathogens. They are recognized by pattern recognition receptors of the innate immune system and represent an important molecular trigger in the development of sepsis [16]. Until the early 1980s, Gram-negative bacteria were proposed as the main inducers of sepsis; however, the incidence of sepsis caused by Gram-positive bacteria and fungi has steadily increased in recent years [6].

Gram-Negative Bacteria

The cell wall of Gram-negative bacteria consists of a thin peptidoglycan layer and an additional outer mem-

brane composed of lipopolysaccharide (LPS), phospholipids and proteins. LPS (in particular the lipid A moiety) acts as an endotoxin and is responsible for the inflammatory response observed during endotoxic shock [17]. Upon its release, LPS activates complement, associates with serum LPS-binding protein and binds to the membrane receptor CD14 and the accessory molecule MD2, inducing TLR4 signaling. Bacteria-derived flagellin stimulates TLR5 [18], whereas peptidoglycan derivatives from Gram-negative bacteria are recognized by complement [19] as well as cytosolic nucleotide-binding oligomerization domain receptor [20]. Furthermore, unmethylated CpG DNA from Gram-negative and Gram-positive bacteria leads to TLR9 activation and plays an important role during infection with Gram-negative bacteria [21].

Gram-Positive Bacteria

The cell walls of Gram-positive bacteria mainly contain peptidoglycan with alternating N-acetylglucosamine and N-acetylmuramic acid, forming a mesh-like

Fig. 2. DAMP-associated changes in organ function and resulting clinical signs. ALI = Acute lung injury; ARDS = acute respiratory distress syndrome; HMV = heart minute volume.

layer surrounding the cytoplasmic membrane. Cell wall PAMPs from Gram-positive bacteria, including lipoteichoic acid, lipoproteins and peptidoglycan, are recognized by complement and TLR2 [19, 22]. In addition, peptidoglycan derivatives are also able to activate cytosolic nucleotide-binding oligomerization domain-2 and the NACHT, LRR and PYD domains-containing protein 1 (NALP1) inflammasome, triggering a potent inflammatory response [23].

Fungi

In addition to dectin-1, pentraxin, mannose and various complement receptors, TLR2 and TLR4 have been implicated in innate immune responses towards various fungal pathogens and PAMPs structurally involved in the fungal cell surface [24]. In particular, the yeast cell wall

component zymosan and *Candida albicans*-derived mannan lead to TLR2/6 and TLR4 activation, respectively [24]. Hereby, two different classes of pattern recognition receptors cooperate closely with each other, as the mannose receptor or the lectin receptor dectin-1 on phagocytes induces pathogen internalization, whereas intracellular TLRs trigger the inflammatory response via activation of NF- κ B [25].

Viruses

Although bacteria are the most common cause of the development of sepsis, viruses can also be involved in such infectious processes. Viruses exhibit several structurally diverse molecular patterns, including surface glycoproteins as well as immunostimulatory nucleotides from the virion itself or produced during viral replica-

tion. Whereas the viral nucleotides are recognized by intracellular receptors, virus-specific glycoproteins are detected by membrane-associated TLR2 and TLR4 [17, 26]. In detail, viral DNA is recognized by TLR9 and DNA-dependent activator of interferon (IFN)-regulatory factors, while single-stranded RNA activates TLR7 and TLR8. Double-stranded RNA and 5'-triphosphate RNA are detected by RIG-I-like receptors, TLR3 and the protein kinase receptor [26, 27].

Endogenous DAMPs in Sepsis

In addition to exogenous PAMPs, the host immune system faces endogenous danger-associated molecular patterns. These can be passively released during sepsis-associated ischemia and necrosis or actively secreted as endogenous alarm signals to warn the host of danger.

DNA

Historically, DNA was considered to be immunologically inert. Today it is well known that DNA can be recognized by the innate immune system. Autologous DNA-chromatin complexes have been shown in vitro to stimulate proinflammatory cytokine release from endothelial cells and splenocytes [28]. TLR9 appears to be responsible for these stimulatory effects of exogenous as well as endogenous DNA [29], as this receptor recognizes not only unmethylated DNA sequences of microbes but also some similar sequences that have a very limited presence in mammalian DNA. There is some evidence indicating the presence of sensors for released cytosolic DNA that may initiate innate immune responses. Takaoka et al. [30] proposed DNA-dependent activator of IFN-regulatory factors as being a cytoplasmic recognition receptor for double-stranded DNA, leading to type I IFN gene expression by the induction of IFN-regulatory factor 3 and the TANK-binding kinase 1 pathway. Investigations of plasma DNA levels of critically ill patients revealed significantly higher DNA levels in those patients who developed sepsis compared to patients who did not and an overall negative correlation with survival [31].

Histones

Xu et al. [32] found that histones released extracellularly in response to inflammatory processes are involved in sepsis-induced endothelial dysfunction, organ failure and death. Activated neutrophils are regarded as a possible source of histones, as they were found to generate neutrophil extracellular traps during sepsis [33]. Neutro-

phil extracellular traps are networks of extracellular fibers, primarily composed of neutrophil DNA and antimicrobial proteins which are capable of killing pathogens extracellularly independently of phagocytic uptake [34]. Another source might be derived from increased apoptosis, necrosis or severe tissue injury overwhelming the phagocytic system and subsequently permitting histones to enter the bloodstream [35]. In mice, the administration of a sublethal dose of histones resulted in neutrophil margination and accumulation, vacuolated endothelium and intra-alveolar hemorrhage [32]. Antibodies against histone 4 reduced the mortality in LPS-, TNF- α - and cecal ligation and puncture (CLP)-induced models of murine sepsis [32].

Nucleophosmin

Nucleophosmin (NPM) is a ubiquitously expressed phosphoprotein belonging to the nucleoplasmin family of chaperones [36]. NPM is mainly localized within the granular regions of the nucleolus, although portions of the protein shuttle between the nucleus and cytoplasm during the cell cycle [37]. NPM is involved in various cellular processes, including histone assembly, ribosome biogenesis, cell cycle progression, apoptosis and cell differentiation. Although the role of NPM in various cellular processes is well characterized, little is known about its role during sepsis. Nawa et al. [38] demonstrated for the first time that NPM exhibits a dual function similar to the high-mobility group box 1 (HMGB1) protein. With regard to its regulatory role during cell homeostasis, NPM is released into the extracellular space from RAW264.7 cells after LPS stimulation. Furthermore, NPM was found in a CLP-induced sepsis model in rats and triggered intercellular adhesion molecule-1 expression on the surface of human umbilical vein endothelial cells. In addition, NPM induced the release of cytokines such as TNF- α , IL-6 and monocyte chemoattractant protein-1 via different mitogen-activated protein kinase (MAPK) pathways in RAW264.7 cells, indicating that NPM release from necrotic or activated cells might play an important role in the induction and maintenance of systemic inflammatory processes during sepsis.

HMGB1

HMGB1 is a nuclear binding protein that facilitates gene transcription by stabilizing nucleosome formation [39]. It can be actively released from leukocytes and dendritic cells upon LPS stimulation, resulting in the production of proinflammatory mediators [40]. In addi-

tion, HMGB1 can be passively released from necrotic or damaged cells [41]. HMGB1 is recognized by TLR2/TLR4 and the cellular receptor for advanced glycation end products, inducing different protein kinase pathways [c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinases 1/2 (ERK1/2), p38 MAPK] and the activation of NF- κ B [40, 42]. In endothelial cells, HMGB1 activates the expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 [43], increasing the permeability of the endothelium and permitting bacterial translocation through endothelial barriers. The importance of HMGB1 in sepsis was originally indicated by its detection in murine models of endotoxemia or CLP [40, 44]. Serum HMGB1 was first detectable 8 h after endotoxin administration and was significantly increased from 16 to 32 h after endotoxin infusion [40]. In clinical settings, HMGB1 levels were significantly elevated in the serum of critically ill patients with sepsis compared to healthy volunteers and correlated negatively with survival [40]. In experimental sepsis, treatment with anti-HMGB1 antibodies administered as late as 24 h after CLP has been proposed to significantly increase the survival rate of mice [45]. Of note, even such a late administration of anti-HMGB1 rescued animals that had already developed clear signs of sepsis, indicating a potentially wide window for therapeutic intervention [46].

Mitochondria as 'the Enemies Within'

According to the endosymbiont hypotheses, mitochondria are evolutionarily derived from α -proteobacteria [47]. Therefore, mitochondria still bear many morphological and biochemical characteristics of their ancestors such as a circular genome containing nonmethylated CpG motifs and the ability to form N-formylated peptides (NFPs) [48]. Therefore, it is not surprising that mitochondria released into the circulatory system lead to activation of the innate immune system similar to the pathways following PAMP exposure. Indeed, intravenous injection of mitochondrial DNA and NFPs in rats resulted in systemic inflammation, suggesting that the body's immune response induced by mitochondrial components mimics the immune response of bacterial sepsis [49]. In addition, plasma mitochondrial DNA concentrations in major trauma patients were several thousand times higher than in healthy controls [49], indicating a damage-induced release of NFPs. Similar to their bacterial ancestors, mitochondria-derived mitochondrial DNA and NFPs are recognized by TLR9 [50] or high-affinity formyl peptide receptors [51], respectively. Thereby,

they induce the phosphorylation of p38, p44 and p42 MAPKs, resulting in neutrophil chemotaxis and the release of inflammatory mediators [49, 51].

Adenosine Triphosphate

Extracellular adenosine triphosphate (ATP) may be considered a further DAMP that can be released from cells or mitochondria upon cellular damage [52]. In addition, certain secretory organelles store large amounts of ATP that are secreted as a danger alarm signal [53]. ATP triggers macrophages, neutrophils and dendritic cells via the P2RX7 receptor to assemble the NALP3 inflammasome, resulting in activation of IL-1 β and IL-18 and release of IL-1 α [54, 55]. IL-1 β exhibits some pyrogenic function and is a key player in the processes of inflammation. In turn, IL-1 β and IL-1 α can stimulate IL-1 receptor signaling, resulting in the generation of proinflammatory cytokines and chemokines, further stimulating the innate immune response.

Danger Translation and Response

The exposure of the host to endogenous as well as exogenous danger molecules leads to activation of a variety of recognition receptors such as TLRs and complement receptors expressed on monocytes, macrophages, dendritic cells and neutrophils as a first line of defense. In general, the activation of these immune cells results in the production of various proinflammatory cytokines and/or chemokines, including TNF- α , IL-1 β , IL-6 and IL-8, lipid mediators, oxygen radicals and tissue-damaging enzymes [56]. A main danger sensing system is presented by the complement system [57].

Complement uses the 'pattern recognition' strategy via C1q and mannose-binding lectin, which both recognize pathogen-derived structures. In addition, the lack of host-specific complement-regulatory proteins leads to induction of the complement system [58]. Excessive complement activation is regarded as a central trigger for the development of sepsis [59, 60]. In the clinical setting, increased plasma concentrations of the complement activation products C3a and C5a were associated with poor outcome [61]. In particular, the anaphylatoxin C5a exhibits various detrimental effects on the host's immune system (e.g. neutrophil dysfunction and immunoparalysis, thymocyte apoptosis, coagulopathy and multiorgan failure) [62], whereas the blockade of C5a or C5a-C5aR interaction improved cellular and organ function and survival in experimental murine sepsis [60].

To efficiently respond to danger, complement synergistically cooperates with TLRs [63] and the coagulation system [64], all of which are involved in the initiation and progression of the inflammatory response in sepsis. For example, complement anaphylatoxins enhance TLR signaling at the level of MAPKs, specifically ERK1/2 and JNK [65]. Vice versa, induction of TLRs activates the expression of complement factors and receptors [66]. The systemic administration of TLR agonists into decay-accelerating factor-deficient mice resulted in significantly increased TNF- α , IL-1 β and IL-6 responses [65]. In addition, C5a-induced TLR cross talk might also involve C5L2, as it contributes synergistically with C5aR to the inflammatory response in CLP-induced sepsis [60].

In the course of danger-associated activation of the innate immune response, disseminated intravascular coagulation (DIC) has been recognized as a major complication during sepsis [67]. In response to inflammatory processes, tissue factor is excessively expressed on endothelial cells, monocytes and macrophages, playing a central role in the initiation of DIC. Subsequent thrombin activation leads to intra- and extravascular fibrin formation, followed by platelet dysfunction and the consumption of coagulation factors. DIC then results in microvascular fibrin deposition, often leading to defective microcirculation [68]. Furthermore, activated coagulation proteins such as thrombin, factor Xa and tissue factor VIIa can stimulate cytokine production, which in turn can stimulate the coagulation cascade. Activated platelets can secrete chemokines, express adhesion molecules on the endothelium and promote neutrophil adherence [69].

Like TLRs, the coagulation system cross talks intensively with the complement cascade [64]. Complement activation products amplify coagulation and inhibit fibrinolysis, mainly through C5a, which induces the expression of tissue factor [70] and plasminogen activator inhibitor 1 [71]. In addition, mannose-binding protein-associated serine protease 2 activates both the complement and coagulation cascades, generating thrombin [72]. Vice versa, components of the coagulation system can amplify complement activation. Activated clotting factor XII triggers the classical complement pathway via C1 cleavage [73], whereas thrombin directly cleaves C5, generating biologically active anaphylatoxin C5a [74]. In this regard, a recent study further indicates novel cross talk between the complement and apoptosis systems. Hereby, the apoptosis-inducing factor granzyme B was shown to be capable of generating C3a and C5a independently of the established complement activation pathways [75]. Therefore, the complement, coagulation and

apoptosis cascades interact synergistically with each other, forming a 'serine protease network' which may play an important role in the pathogenesis of sepsis [9].

Conclusion

Recently, there has been considerable progress in identifying novel endogenous DAMPs that are implicated in the pathogenesis of inflammatory diseases such as sepsis. Endogenous DAMPs can be passively released as a result of nonphysiological cell death or actively secreted as alarm signals (alarmins) to warn the host of danger by activating the innate immune response. In this regard, DNA, histones or mitochondria might be useful surrogate markers for the development and progression of sepsis. However, most endogenous DAMPs arise from cellular breakdown due to any kind of cellular damage, which can be the result of severe tissue trauma or various inflammatory processes apart from sepsis. Thus, DAMPs might exhibit potent targets for therapeutic interventions. The administration of HMGB1-neutralizing antibodies or inhibitors has been beneficial in experimental sepsis [46], although the underlying mechanisms are not fully understood. The inflammatory response in sepsis is a complex interplay of different host defense systems and their mediators, such as the complement, TLR and coagulation systems, resulting in a severe dysregulation of the immune-regulatory network. A deeper understanding of the underlying pathways is necessary to provide new insights to modulate the DAMP response and to develop novel management strategies to effectively improve the clinical outcome of sepsis in the future.

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