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Targeting HMGB1 in the treatment of sepsis

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Abstract

Introduction—Sepsis refers to the host's deleterious and non-resolving systemic inflammatory response to microbial infections, and represents the leading cause of death in the intensive care unit. The pathogenesis of sepsis is complex, but partly mediated by a newly identified alarmin molecule, the high mobility group box 1 (HMGB1).

Areas covered—Here we review the evidence that support extracellular HMGB1 as a late mediator of experimental sepsis with a wider therapeutic window, and discuss the therapeutic potential of HMGB1-neutralizing antibodies and small molecule inhibitors (herbal components) in experimental sepsis.

Expert opinion—It will be important to evaluate the efficacy of HMGB1-targeting strategies for the clinical management of human sepsis in the future.

Keywords

innate immune cells; cytokines; sepsis; antibodies; HMGB1; herbal component; PKR

1. Introduction

Cohabitating with various microbes, animals have developed multiple strategies to deal with microbial infections. The epithelial barriers serve as the first layer of defense by limiting the access and/or growth of many pathogens. If they are breached, the host's innate immune system mounts an immediate biological response, termed "inflammation" ("set on fire", in Greek), to confine and remove the invading pathogens [1]. If successful, the inflammatory process resolves to restore immunologic homeostasis; otherwise the invading pathogens can leak into the blood stream, triggering a widespread, systemic inflammatory response termed "sepsis" (Figure 1). Sepsis refers to the host's deleterious and non-resolving systemic inflammatory response to microbial infection [2], and represents the leading cause of death in the intensive care unit. As a continuum of increasing clinical severity, "severe sepsis" is often associated with one or more acute organ dysfunctions [3]. Despite recent advances in antibiotic therapy and intensive care, the overall mortality rate of severe sepsis remains high (28.6%) [4], claiming 215,000 lives annually in the U.S. alone. Current therapies for sepsis are still largely supportive and limited to a few clinical interventions including antibiotics, steroidal anti-inflammatory drugs (e.g., hydrocortisone) and early goal directed therapies (EGDT). Unfortunately, these supportive therapies are often ineffective, prompting the on-going search for novel therapeutic strategies for human sepsis. Here we review the accumulating evidence that support the therapeutic potential of several HMGB1-targeting agents in animal models of sepsis.

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2. Pathogenesis of Sepsis

The pathogenesis of sepsis is rather complex, but partly attributable to dys-regulated systemic inflammatory responses propagated by innate immune cells including macrophages and monocytes. While continuously patrolling the body to search for invading pathogens or damaged tissues, monocytes immediately infiltrate into the infected/injured tissues upon detecting microbial products (termed pathogen-associated molecular patterns, PAMPs) or damage-associated molecular patterns (DAMPs) [5]. Upon reaching extravascular tissues, these monocytes are differentiated into tissue-specific resident macrophages, which are responsible for ingesting and eliminating invading pathogens in alliance with other phagocytes (e.g., neutrophils) [6].

Additionally, macrophages/monocytes are equipped with receptors [such as the Toll-like receptors (TLRs) TLR2, TLR3, TLR4, and TLR9] [7–11] specific for various PAMPs (e.g., bacterial peptidoglycan, ds-RNA, endotoxin, and CpG-DNA) [12;13]. The engagement of PAMPs with respective receptors triggers the sequential release of early (e.g., TNF, IL-1 and IFN-γ) and late (e.g., HMGB1) proinflammatory mediators (Figure 1) [14–16]. Early mediators, individually or in combination, contribute to the pathogenesis of lethal systemic inflammation. For instance, neutralizing antibodies against an early cytokine, TNF [17], reduce lethality in animal models of endotoxemic/bacteremic shock. However, the early kinetics of systemic TNF accumulation makes it difficult to target in clinical settings [17], prompting a search for other late mediators (e.g., HMGB1) that may offer wider therapeutic windows.

3. HMGB1 as a late mediator of experimental sepsis

HMGB1 is constitutively expressed to maintain a large "pool" of pre-formed protein in the nucleus of most cells [18;19]. It contains two internal repeats of positively charged domains ("HMG boxes" known as "A box" and "B box") in the N-terminus, and a continuous stretch of negatively charged (aspartic and glutamic acid) residues in the C-terminus. These HMG boxes enable HMGB1 to bind chromosomal DNA, and fulfill its nuclear functions such as maintaining the nucleosomal structure and stability and regulating gene expression [20].

3.1. Active Release

In response to PAMPs (e.g., ds-RNA, CpG-DNA and endotoxin) [16;21], DAMPs (e.g., ATP) [22], or cytokines [e.g., interferon (IFN)-γ] [23], macrophages/monocytes actively release HMGB1 in a dose- and time-dependent manner (Figure 1). Lacking a leader signal sequence, HMGB1 cannot be actively secreted via the classical ER-Golgi secretory pathway [16]. Instead, activated macrophages/monocytes acetylated HMGB1 at the nuclear localization sequences, leading to sequestration of HMGB1 within cytoplasmic vesicles and extracellular release [19;23;24]. Accumulating evidence has suggested an essential role for inflammasome activation in the regulation of LPS/ATP-induced HMGB1 release [22;25], because genetic disruption of key inflammasome components (e.g., caspase 1 or Nalp3) completely impaired the LPS/ATP-induced HMGB1 release. Furthermore, the double-stranded RNA-activated protein kinase R (PKR) functions as the key regulator of the inflammasome activation and HMGB1 release (Figure 2) [22].

Interestingly, ultra-pure LPS (*free from contaminating bacterial proteins and nucleic acids*) fails to trigger HMGB1 release unless the initial LPS (10 μg/ml) priming is accompanied by a second stimulus (e.g., ATP) [22;25]. Similarly, ATP itself is unable to induce HMGB1 release without prior LPS exposure [25], even though it can induce PKR phosphorylation [22] and inflammasome activation [26–28]. In contrast, prolonged stimulation with crude LPS (*containing trace amounts of bacterial proteins and nucleic acids*) triggered dramatic

HMGB1 release [16], as well as an up-regulation of PKR expression (> 2-folds) and phosphorylation (> 8-fold) in macrophages. It is plausible that the crude LPS activates inflammasomes initially through triggering the release of ATP, which then binds to the purinergic $P2X_7$ receptor ($P2X_7R$) [29], thereby inducing feed-forwarding ATP release, and subsequent inflammasome activation (Figure 2) [26–28].

3.2. Passive Leakage

In addition, HMGB1 can be passively released from damaged cells [30] following sterile tissue injury resulting from ischemia/reperfusion [31–33], non-penetrating trauma [34–36], or exposure of toxic chemicals to the liver [37–41]. As a DAMP, extracellular HMGB1 passively released by necrotic cells allows innate immune cells to respond to sterile injury (Figure 1) [42;43]. In fact, the sterile tissue injury-elicited systemic inflammatory response syndrome (SIRS) is indistinguishable from microbial infection-induced sepsis [2;44]. Thus, seemingly unrelated conditions such as infection and injury can converge on a common process - inflammation [42], which is orchestrated by HMGB1 and other proinflammatory mediators (e.g., mitochondrial DNA) released both by activated innate immune cells and by damaged tissues [42–45] (Figure 1). Similarly, HMGB1 can be passively released by cells infected by various viruses (e.g., West Nile, Salmon anemia, Dengue, and influenza viruses) [46–48] or mycobacteria [49;50], suggesting HMGB1 as a pathogenic mediator of viral infection-elicited inflammatory diseases [51].

3.3. Extracellular HMGB1 as an alarmin

Once released, extracellular HMGB1 functions as an alarmin signal to alert, recruit, and activate innate immune cells [52;53]. For instance, HMGB1 is capable of stimulating the migration of monocytes, dendritic cells [54;55] and neutrophils [56], functioning as a chemokine to facilitate the recruitment of innate immune cells to the sites of infection or injury [57;58]. Furthermore, HMGB1 binds to various PAMPs (e.g., CpG-DNA or LPS), thereby facilitating their recognition by respective receptors [59], and consequently augmenting the PAMPs-induced inflammatory responses [59]. In addition, HMGB1 can bind to multiple cell surface receptors including RAGE [59], TLR2, TLR4 [60–62], TLR9 [21;59], cluster of differentiation 24 (CD24)/Siglec-10 [63], Mac-1 [56], thrombomodulin [64], as well as single transmembrane domain proteins (e.g., syndecans) [65]. Consequently, it can activate macrophages [60;66–69] and endothelial cells [70;71] to produce proinflammatory cytokines, chemokines, and adhesion molecules.

Intriguingly, recent studies have suggested that chemical modification may affect the cytokine or chemokine activities of HMGB1. For instance, it was shown that reactive oxygen species (ROS) may oxidize HMGB1 to form intra-molecular disulfide bond between thiol group of Cys^{106} and Cys^{23} or Cys^{45}, and consequently abolish HMGB1-mediated immunostimulatory activities [72]. Furthermore, it has been suggested that only the fully reduced ("all-thiol") HMGB1 has chemokine-like activities; the Cys^{23}-Cys^{45} disulfide form has cytokine activities; whereas the fully oxidized HMGB1 lost both chemokine and cytokine activities [73–75]. Considered together, these studies suggest extracellular HMGB1 as an alarmin signal to recruit, alert, and activate innate immune cells, thereby sustaining a potentially injurious inflammatory response during sepsis.

3.4. Pathogenic role of HMGB1 in sepsis

Experimentally, sepsis is routinely induced by several techniques including the infusion of exogenous bacterial toxins (endotoxemia), as well as the disruption of host epithelial barrier to induce microbial translocation (such as the cecal ligation and puncture, CLP). Each model has particular strengths and weaknesses with respect to their ability to mimic the clinical progression of human sepsis [76]. Endotoxemia is generally considered as a model of septic

shock, and has been widely used to investigate the roles of various cytokines in lethal systemic inflammation. In contrast, CLP allows bacteria spillage into the peritoneal cavity, mimicking the human clinical conditions of perforated appendicitis or diverticulitis. The severity of sepsis, as reflected by the eventual mortality rates, can be controlled surgically by varying the size of the needle used for cecal puncture. Thus, the CLP model is considered as the most clinically relevant experimental model for human sepsis [77].

In murine models of endotoxemia and CLP-sepsis, HMGB1 is first detected in the circulation eight hours after the disease onset, and subsequently increased to plateau levels from 16 to 32 hours [16;78]. This late appearance of circulating HMGB1 parallels with the onset of animal lethality from endotoxemia or sepsis, and distinguishes itself from TNF and other early proinflammatory cytokines [79]. The pathogenic role of HMGB1 in endotoxemia was inferred from studies using HMGB1-neutralizing antibodies, which conferred a dose-dependent protection against endotoxin-induced tissue injury and lethality [16;80]. In a more clinically relevant animal model of sepsis (induced by CLP), delayed administration of HMGB1-specific neutralizing antibodies beginning 24 h *after* CLP, dose-dependently rescued rodents from lethal sepsis [78;81;82]. Taken together, these experimental data establish extracellular HMGB1 as a critical late mediator of experimental sepsis, which can be therapeutically targeted within wider therapeutic windows than other early cytokines.

4. Therapeutic potential of HMGB1-inhibiting agents

Currently, there is no effective therapy for the treatment of sepsis, although a number of interventions are routinely employed in clinical settings. For instance, appropriate broad-spectrum antibiotics are often given to patients to facilitate the elimination of bacterial pathogens [3]. However, the disruption of bacteria may be accompanied by the liberation of PAMPs (such as endotoxin or CpG-DNA) that adversely stimulate innate immune cells to produce proinflammatory cytokines. Thus, various anti-inflammatory steroids (such as hydrocortisone, methylprednisolone, dexamethasone, fludrocortisone) are frequently used to modulate the excessive inflammatory response, despite the lack of reproducible efficacy in the treatment of human sepsis [83–85]. As a supportive intervention, the 'early goal directed therapy' employs extremely tight control of a number of physiological parameters (such as central venous pressure, mean arterial blood pressure, central venous oxygen saturation, and hematocrit) with discrete, protocol driven interventions of crystalloid fluid, vasopressors, and blood transfusions. It is not yet conclusive whether this simple intervention significantly reduces the mortality of patients with sepsis or septic shock [86;87], prompting the search for HMGB1-targeting agents for the treatment of human sepsis.

Since our seminal discovery of HMGB1 as a late mediator of lethal endotoxemia [16], a growing list of agents has been tested for activities in inhibiting HMGB1 release, and efficacy for protecting against lethal endotoxemia or sepsis (Table 1). The HMGB1-inhibiting agents range from intravenous immunoglobulin (IVIG) [88], anti-coagulant agents (antithrombin III, thrombomodulin, danaparoid sodium) [64;89], acute phase proteins (e.g., fetuin-A) [90], endogenous hormones (e.g., insulin, vasoactive intestinal peptide, ghrelin) [91;92;92;93], to endogenous small molecules (e.g., acetylcholine, stearoyl lysophosphatidylcholine, glutamine) [18;94–96]. In addition, a number of herbal extracts (e.g., Danggui, Mung bean, and Prunella vulgaris) [97–99] and components (e.g., nicotine, EGCG, tanshinone, glycyrrhizin, chlorogenic acid, Emodin-6-O-β-D-glucoside, Rosmarinic acid, isorhamnetin-3-O-galactoside, Persicarin, Forsythoside B, chloroquine, acteroside) [100–111] have been proven effective in inhibiting endotoxin-induced HMGB1 release (Figure 3). Nevertheless, various herbal components appear to utilize distinct mechanisms to prevent HMGB1 release by activated macrophages/monocytes. For instance, a major green tea component, EGCG, prevents the LPS-induced HMGB1 release strategically by

destroying it in the cytoplasm via a cellular degradation process – autophagy [112]. In contrast, a derivative of tanshinone IIA, TSN-SS selectively inhibits HMGB1 release by facilitating endocytosis of exogenous HMGB1, leading to subsequent degradation via a lysosome-dependent pathway [113]. A pannexin-1 channel blocker, carbenoxolone (CBX), attenuates LPS-induced HMGB1 release by preventing the expression and phosphorylation of PKR, a newly identified regulator of inflammasome activation and HMGB1 release (Figure 2) [22;114].

In light of the capacity of herbal ingredients in preventing endotoxin-induced HMGB1 release, we explored their efficacy in animal models of lethal endotoxemia. Consistent with previous report [115;116], we found that the intraperitoneal administration of EGCG (4.0 mg/kg) at −0.5, +24, and +48 h post onset of endotoxemia significantly improved animal survival from 50% to 76% [101]. To further explore its therapeutic potential, we employed the clinically relevant animal model of CLP-induced sepsis. Given the late and prolonged kinetics of HMGB1 accumulation in experimental sepsis [78], the first dose of EGCG was given 24 h after the onset of sepsis - a time point when mice developed clear signs of sepsis including lethargy, diarrhea, and piloerection. Repetitive intraperitoneal administration of EGCG (at 24, 48, and 72 h post CLP) significantly increased animal survival rates from 53% to 82% [101]. Even when given orally, EGCG still rescued mice from lethal sepsis, significantly increasing animal survival rates from 16% to 44% [112]. As predicted, delayed administration of EGCG did not affect the circulating levels of early cytokines, but significantly attenuated systemic accumulation of HMGB1 [101]. Furthermore, it attenuated circulating levels of IL-6 (by 75%) and KC (by 60%) - two reliable surrogate markers of lethal sepsis [117;118]. Intriguingly, we found that EGCG facilitated bacterial elimination in selective organs (e.g., the liver and lung) in an animal model of sepsis [111]. It is not yet known whether these antibacterial properties are attributable to the possibilities that EGCG directly kill microbes by altering microbial protein conformations and functions [111], or indirectly by modulating macrophage-associated innate immune responses. Considered together, these experimental data indicate that EGCG protects mice against lethal sepsis partly by attenuating systemic HMGB1 accumulation, and partly by facilitating bacterial elimination.

Similarly, repeated administration of CBX or TSN-SS beginning 24 h *after* the onset of sepsis (followed by additional doses at 48 and 72 h post CLP) conferred a dose-dependent and significant protection against lethal sepsis [102;119], supporting a therapeutic potential for herbal components in experimental sepsis. Furthermore, both CBX and TSN-SS significantly reduced HMGB1 levels not only systemically in the circulation [102], but also locally in the peritoneal lavage fluid, suggesting that CBX and TSN-SS confers protection against lethal sepsis possibly by attenuating local and systemic HMGB1 accumulation. In addition, a growing list of HMGB1 inhibitors has been proven protective by attenuating systemic HMGB1 release or action (Table 1), stimulating further interests in possible future clinical studies.

5. Conclusions

5.1 Sepsis is the host's deleterious and non-resolving systemic inflammatory response to microbial infection, and is partly mediated by various proinflammatory mediators (e.g., HMGB1).

5.2 HMGB1 is secreted by activated macrophages/monocytes through complex mechanisms dependent on the activation of PKR and inflammasomes.

5.3 HMGB1 can also be passively released by necrotic cells following ischemia-reperfusion, trauma, and injury, thereby functioning as a DMAP molecule to orchestrate the injury-elicited inflammatory responses.

5.4 Extracellular HMGB1 functions as an alarmin signal to alert, recruit, and activate innate immune cells, thereby serving as a late mediator of lethal sepsis with a wider therapeutic window.

5.5 A number of endogenous macromolecules (e.g., intravenous immunoglobulin, anti-coagulants, acute phase proteins, and hormones) or small molecules (e.g., acetylcholine, stearoyl lysophosphatidylcholine, glutamine) have been found to be effective in inhibiting HMGB1 release, and protecting against lethal endotoxemia or sepsis.

5.6 Many herbal components have been proven effective in inhibiting HMGB1 release through divergently distinct mechanisms, ranging from inducing autophagic degradation, stimulating endocytic uptake, to preventing PKR activation.

5.7 Many herbal extracts and components have been proven protective in animal models of experimental sepsis.

5.8 Unlike neutralizing antibodies, the herbal components may confer protection against lethal sepsis by inhibiting HMGB1 as well as other potential off-targets. For instance, at the doses that completely prevented HMGB1 release, TSN-SS also partially inhibited endotoxin-induced release of nitric oxide, IL-1α (by $50 \pm 7\%$), platelet factor 4 (PF-4, by $35 \pm 6\%$) and MCP-5 (by $25 \pm 5\%$) [102], although it did not affect most other cytokines (e.g., TNF, IL-6, IL-12) and chemokines (e.g., KC, MCP-1, MIP-1α, MIP-2).

6. Expert opinion

For complex systemic inflammatory syndromes, it might be difficult to translate successful animal studies into clinical applications. For instance, various therapeutic strategies against bacterial endotoxin including neutralizing antibodies [120] and binding proteins [121] failed to improve survival in sepsis clinical trials. Similarly, agents blocking endotoxins from binding to the TLR4 receptor (e.g., Eritoran) also did not show benefit in a recent clinical trial [122], raising questions about the feasibility of TLR4-blocking agents in the treatment of human sepsis. Notably, the innate recognition system [consisting of LPS-binding protein (LBP), CD14, and TLR4] is essential for macrophages/monocytes to effectively recognize and respond to minute amounts of endotoxins. If presented at high levels, endotoxins can be internalized into macrophage cytoplasmic compartments, thereby activating the release of inflammasome-dependent cytokines via TLR4-independent signaling pathways [123;124]. Thus, if endotoxins reach critical concentrations during sepsis, aberrant cytoplasmic localization may occur to trigger inflammasome-dependent release of HMGB1 and other pathogenic mediators in TLR4-independent mechanisms. There, it may be important to use detectable endotoxemia as a precondition for enrollment of septic patients in respective TLR4-targeting sepsis trials.

Although neutralizing antibodies against early cytokines (e.g., TNF) [17;125] are protective in animal models of endotoxemia or bacteremia, these agents also failed in sepsis clinical trials [126]. This failure partly reflected the complexity of the underlying pathogenic mechanisms of sepsis, and the consequent heterogeneity of the patient population [1;3]. It may also be attributable to pitfalls in the selection of non-feasible therapeutic targets or non-realistic clinical outcome measures (such as survival rates) [1]. Nevertheless, the investigation of pathogenic cytokines in animal models of diseases has led to the

development of successful cytokine-targeting therapeutic strategies. For instance, chimeric anti-TNF monoclonal antibody (infliximab) and a soluble TNF receptors-Fc fusion protein (sTNF-R-Fc, etanercept) have been approved for patients with debilitating chronic inflammatory diseases, such as rheumatoid arthritis (RA) [127]. It is thus necessary and hopeful to continue the search for clinically feasible therapeutic target and drugs for clinical management of human sepsis.

The discovery of HMGB1 as a late mediator of experimental sepsis has stimulated a great interest in developing neutralizing antibodies for clinical management of human sepsis. In the animal model of sepsis, monoclonal antibodies effectively rescued mice from the lethal sequelae even when given 24 h post the onset of sepsis. It is not yet known whether HMGB1 can ever be a clinically feasible therapeutic target for human sepsis until HMGB1-neutralizing antibodies are tested in clinical trials. Humanized anti-HMGB1 antibodies have been commercially developed by MedImmune, but the company has decided not to conduct sepsis clinical trials after strategic merge with AstraZeneca in 2007. In addition to business-related reasons, it may also relate to the frustrating difficulties encountered by the MedImmune while attempting to develop reliable bioassays for screening HMGB1-neutralizing antibodies. Specifically, the cytokine or chemokine activities of recombinant HMGB1 protein were found to be paradoxically unstable. Subsequently, it was discovered that the redox status of HMGB1 protein affected its cytokine- or chemokine-like activities in a dramatically divergent fashion [73–75]. Of course, the failure of previous sepsis trials may have similarly discouraged many investigators from believing that a single therapeutic target will ever be feasible for a complex syndrome like sepsis. Moreover, many investigators believe that some patients with sepsis die from immunosuppression rather than exaggerated inflammation, even though the excessive inflammation may still be a prerequisite of the subsequent immunosuppression. Interestingly, it has been shown that HMGB1 can: i) enhance the regulatory T cell-mediated immunosuppression [128;129]; ii) induce immunological tolerance [72;130;131]; and iii) impair phagocytic elimination of microbes [132;133] and apoptotic cells [134]. Given the relative wider therapeutic window for HMGB1 in experimental sepsis, it is important to pre-determine circulating HMGB1 levels in their circulation before anti-HMGB1 therapy is given to septic patients. Currently, humanized anti-HMGB1 antibodies are being developed by major pharmaceutical companies to treat inflammatory autoimmune diseases (such as lupus). Future clinical studies are expected in the coming years to test the efficacy of HMGB1-neutralizing antibodies in the clinical management of human inflammatory diseases.

Notably, humanized monoclonal antibodies (mAb) are manufactured in low-yield and time-consuming mammalian cells, and are thus tremendously more expensive than developing small molecule chemical drugs. For example, the recommended dose for frequent injections of Humira (TNF mAb) to treat rheumatoid arthritis is 40 mg every two weeks, totaling > 1 gram (> $16,000) per year. It is thus essential to develop cost-effective small molecule drugs for the clinical management of human sepsis. We and others have found a number of small molecules that effectively prevented HMGB1 release, and conferred protection against lethal endotoxemia and sepsis. Extensive pre-clinical toxicology and safety studies are needed before testing these HMGB1 inhibitors for efficacy in clinical studies.

Will any HMGB1 inhibitors also become therapeutic agents for human sepsis? One of the most selective HMGB1 inhibitor, TSN-SS, has already been used in China as a medicine for patients with cardiovascular disorders [135]. Even in septic animals, TSN-SS reduced total peripheral vascular resistance, and yet increased cardiac stroke volume and cardiac output [102]. The dual effects of TSN-SS in attenuating late inflammatory response and improving cardiovascular function make it a promising therapeutic agent for sepsis. The capacity to facilitate endocytic HMGB1 uptake by professional phagocytes may provide basis for the

treatment of both infection- and injury-elicited inflammatory diseases. Several lines of evidence have supported the feasibility of developing TSN-SS as potential therapies for human sepsis. These include the lack of drug toxicity in the clinical management of other cardiovascular diseases with TSN-SS; and the effectiveness of TSN-SS in reducing lethality in animal models of sepsis. The advantages of TSN-SS over many other sepsis drugs in development are: 1) fewer side effects are expected, because it has been prescribed in China as a cardiovascular drug for many years; and 2) the low cost of purification of this drug should reduce sepsis therapy costs significantly, enabling treatments to become more accessible. Of course, it is not yet known whether a better protection could be achieved by combinational therapy with several anti-HMGB1 agents. It is thus important to further explore the therapeutic potential of these HMGB1-inhibiting agents in future studies.

Acknowledgments

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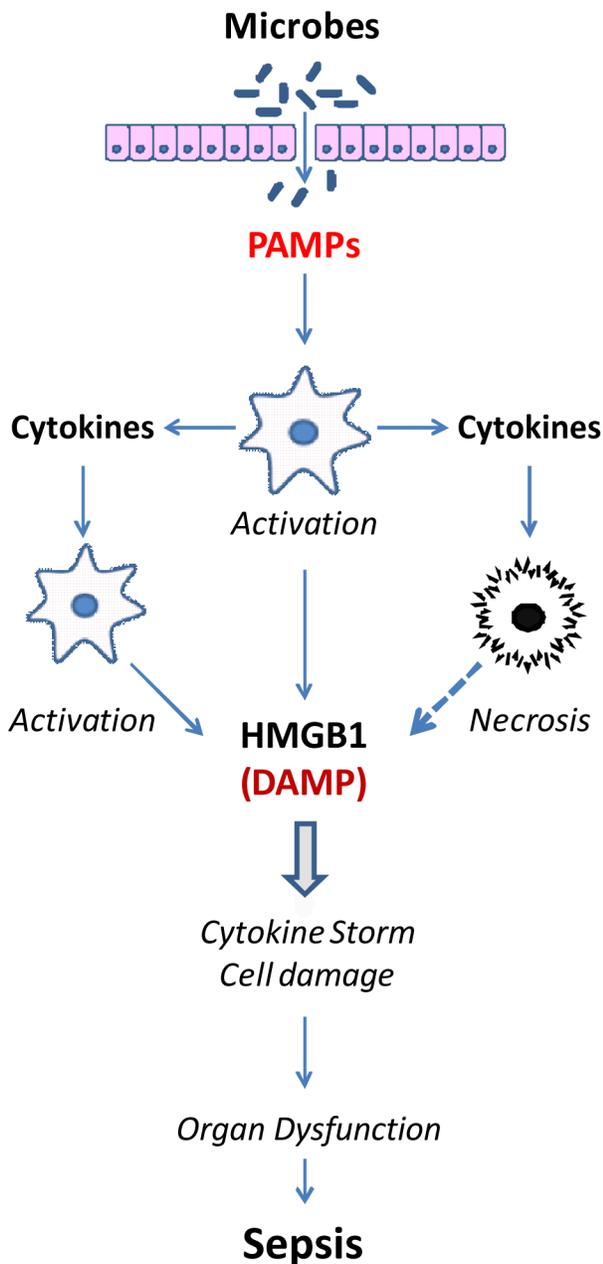
Figure 1. A microbial infection can trigger a systemic inflammatory response
The disruption of epithelial barrier allows invasion of microbial pathogens, which elicit an innate immune response through liberating pathogen-associated molecular patterns (PAMPs). In immune-compromised hosts, the excessive accumulation of PAMPs causes cytokine storm and accompanying cell injury during the early stage of sepsis. Subsequently, damage-associated molecular patterns (DAMPs, such as HMGB1, mtDNA, and ATP) are released from injured tissue, and further amplify the cytokine storms and cell damages, resulting in organ dysfunction and sepsis.
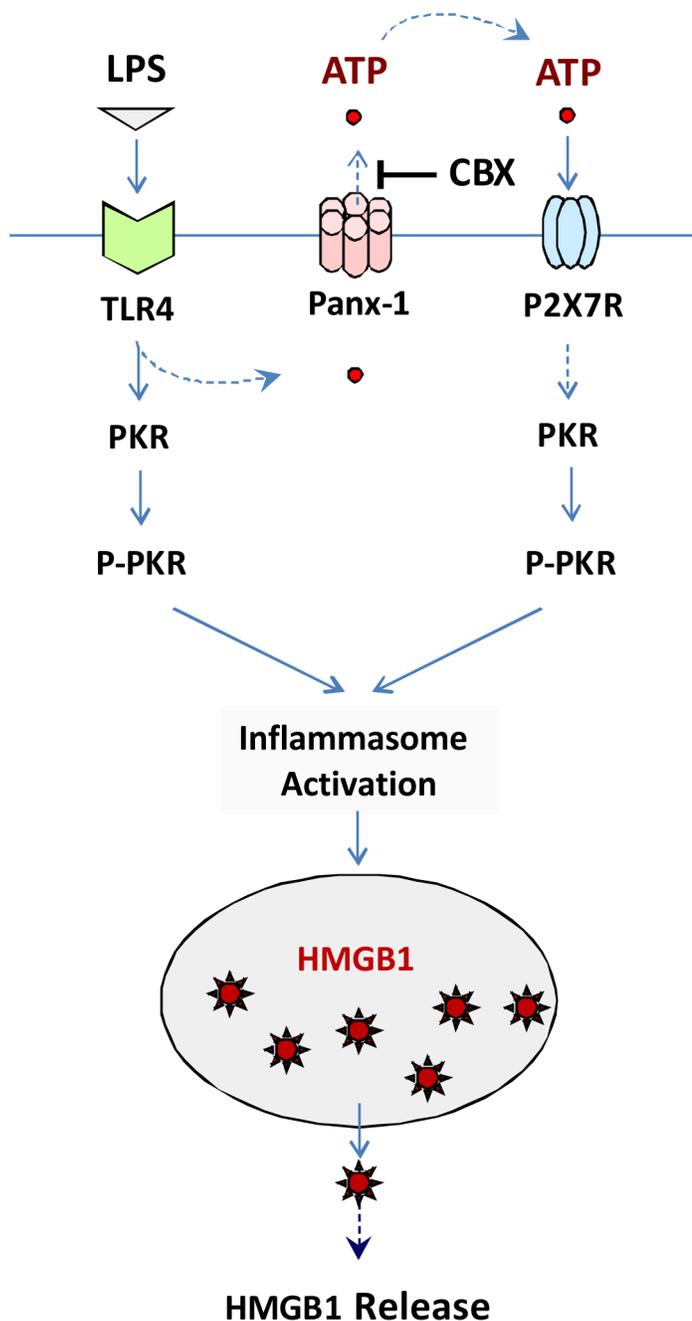
Figure 2. CBX inhibits LPS-induced HMGB1 release by preventing PKR activation
Prolonged stimulation with crude LPS may lead to panx-1 hemichannel-mediated ATP efflux, and up-regulation of PKR expression. Extracellular ATP then binds to $P2X_7R$ and activates the ATP-gated $P2X_7R$ and panx-1 channels, leading to PKR phosphorylation and subsequent inflammasome-dependent HMGB1 release. CBX may block LPS-induced ATP efflux through Panx-1, thereby impairing ATP/$P2X_7R$-mediated PKR activation, and subsequent inflammasome-dependent HMGB1 release.
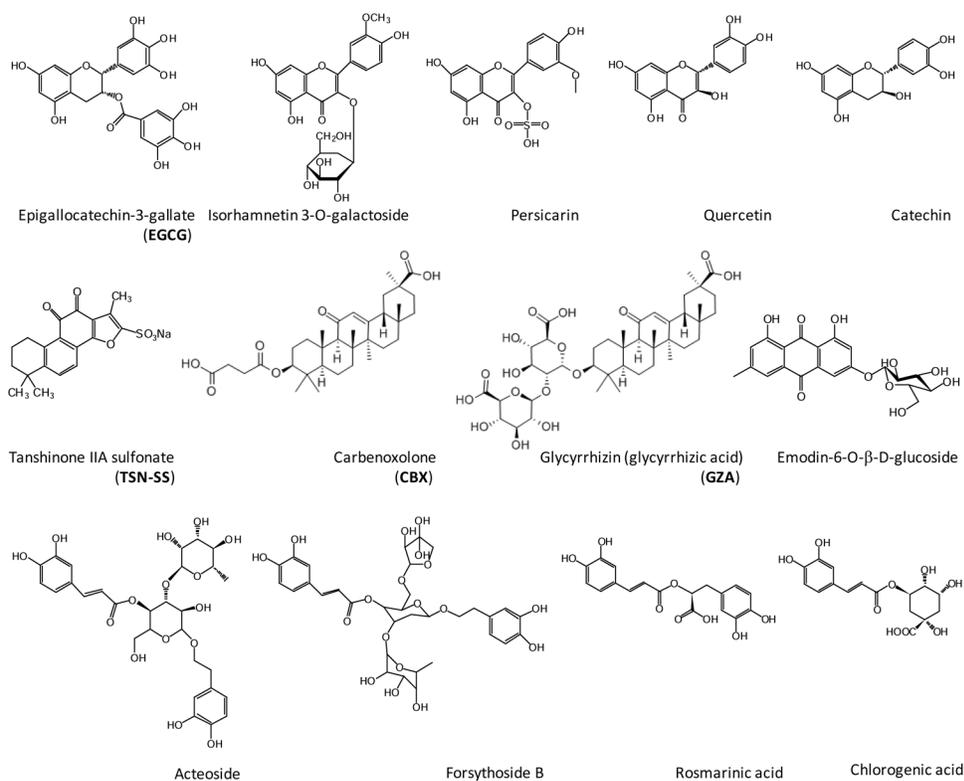
Figure 3.
Chemical structures of HMGB1-inhibiting herbal components.

Table 1

Potential HMGB1-targeting therapeutic agents.

Agents	Models of Sepsis	↓HMGB1
Neutralizing antibodies	Endotoxemia/Sepsis	Activity
Anti-coagulant agents		
Anti-thrombin III	Endotoxemia	Release
Thrombomodulin	Endotoxemia	Activity
Acute phase proteins		
Fetuin-A	Endotoxemia/Sepsis	Release
Endogenous hormones		
Insulin	Endotoxemia	Release
Vasoactive intestinal peptide	Sepsis	Release
Ghrelin	Sepsis	Release
Intravenous immunoglobulin	Sepsis	Release
Stearoyl lysophosphatidylcholine	Endotoxemia/Sepsis	Release
Herbal extract		
Danggui	Endotoxemia/Sepsis	Release
Mung bean	Endotoxemia/Sepsis	Release
Prunella vulgaris	Sepsis	Release
Herbal components		
Nicotine	Endotoxemia/sepsis	Release
Danshen (TSN-SS)	Endotoxemia/sepsis	Release/Activity
Green tea (EGCG)	Endotoxemia/sepsis	Release/Activity
Carbenoxolone	Sepsis	Release
heme-oxygenase-1 (HO-1)-derived carbon monoxide (CO)	Endotoxemia/sepsis	Release
Chloroquine	Endotoxemia/Sepsis	Release
Acteoside, an active phenylethanoid glycoside	Sepsis	Release
Glycyrrhizin	Endotoxemia	Release
Chlorogenic acid	Endotoxemia/Sepsis	Release
Emodin-6-O-β-D-glucoside	Sepsis	Release
Rosmarinic acid (RA)	Endotoxemia/Sepsis	Release
Isorhamnetin-3-O-galactoside	Endotoxemia/Sepsis	Release
Persicarin	Endotoxemia/Sepsis	Release
Forsythoside B	Sepsis	Release