



## REVIEW ARTICLE

## Targeting HMGB1 for the treatment of sepsis and sepsis-induced organ injury

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High mobility group box 1 (HMGB1) is a ubiquitous nuclear protein that is present in almost all cells and regulates the activity of innate immune responses in both intracellular and extracellular settings. Current evidence suggests that HMGB1 plays a pivotal role in human pathological and pathophysiological processes such as the inflammatory response, immune reactions, cell migration, aging, and cell death. Sepsis is a systemic inflammatory response syndrome (SIRS) that occurs in hosts in response to microbial infections with a proven or suspected infectious etiology and is the leading cause of death in intensive care units worldwide, particularly in the aging population. Dysregulated systemic inflammation is a classic characteristic of sepsis, and suppression of HMGB1 may ameliorate inflammation and improve patient outcomes. Here, we focus on the latest breakthroughs regarding the roles of HMGB1 in sepsis and sepsis-related organ injury, the ways by which HMGB1 are released, and the signaling pathways and therapeutics associated with HMGB1. This review highlights recent advances related to HMGB1: the regulation of HMGB1 might be helpful for both basic research and drug development for the treatment of sepsis and sepsis-related organ injury.

**Keywords:** high mobility group box 1; sepsis; LPS; inflammation; Toll-like receptor

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## INTRODUCTION

Detecting pathogens is the first step in mounting an effective immune response to eliminate invading organisms and maintain immune homeostasis [1]. If unsuccessful, invading pathogens may enter the blood stream and trigger widespread systemic inflammation termed sepsis. Sepsis is also referred to as systemic inflammatory response syndrome (SIRS), occurs in response to microbial infections with a proven or suspected infectious etiology, and is the leading cause of death in intensive care units worldwide [2–5]. Notably, sepsis is common in the aging population and disproportionately affects patients with cancer and underlying immunosuppression [6]. The pathogenesis of sepsis is rather complex and partially attributable to dysregulated inflammatory responses propagated by innate immune cells, including macrophages, monocytes, neutrophils [7, 8], which release proinflammatory cytokines, resulting in damage to tissue and organs, metabolic acidosis, hypotension, or even death. To date, there are no effective therapeutics to cure sepsis and sepsis-related SIRS. The current therapies for sepsis in the clinic remain largely supportive and are limited to a few clinical interventions, including antibiotics, steroidal anti-inflammatory drugs, and early

goal-directed therapies [9]. Unfortunately, these treatments are usually ineffective, and most patients still suffer from shock or death [10]. Moreover, some drugs have adverse effects that contribute to poor patient compliance. Accumulating evidence has demonstrated that restoring homeostasis and ameliorating dysregulated systemic inflammation are critical. Thus, a better understanding of the pathophysiological role of proinflammatory cells and cytokines in sepsis is required.

High mobility group box 1 (HMGB1) is a small protein of 215 amino acid residues composed of two tandem high mobility group (HMG) box domains (A box and B box) in the N-terminus and a continuous stretch of negatively charged residues in the C-terminus [11, 12]. HMGB1 is a ubiquitous nuclear protein present in almost all cells that regulates the activity of innate immune responses intracellularly and extracellularly [13]. HMGB1 was originally described as a nuclear DNA-binding protein. HMGB1 is properly defined as a cytokine because it stimulates proinflammatory responses in monocytes/macrophages *in vivo* in standardized models of systemic and local inflammation and mediates delayed endotoxin lethality in animal models of endotoxemia and sepsis [14]. Recently, a better understanding of HMGB1 has been

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gradually obtained. Serum levels of HMGB1 are high in patients who die of sepsis [8], whereas blocking HMGB1 preserves the ability of neutrophils to activate NADPH oxidase in patients recovering from septic shock [15]. Given the emerging roles of HMGB1 in systemic inflammation, the present review aims to discuss recent breakthroughs related to HMGB1 in sepsis and potential therapeutics.

### **PATHOGENESIS OF SEPSIS**

The range of presentations of sepsis syndrome is very wide because sepsis can originate from virtually any infecting organism. The pathogenesis of sepsis is extremely complex and involves the inflammatory response, immune dysfunction, mitochondrial dysfunction, coagulation disorders, neuroendocrine-immune network abnormalities, endoplasmic reticulum stress and autophagy.

#### **Immune dysfunction**

Immune activation in response to infection is triggered by highly conserved microbial pathogen-associated molecular patterns that are recognized by pattern-recognition receptors, including Toll-like receptors, on cells in the innate immune system. This interaction activates the release of both proinflammatory and anti-inflammatory mediators through the activation of nuclear factor  $\kappa$ B (NF- $\kappa$ B) and neutrophils [6]. In addition, cytokines lead to neutrophil-endothelial cell adhesion, complement activation and clotting cascades. Sepsis was traditionally acknowledged as a systemic and proinflammatory response to infection, which subsequently leads to a cascade of immunosuppressive responses, including lymphopenia, anergy, and secondary infections [16]. Moreover, the maturation of dendritic cells (DCs) in the spleen and lymph nodes is impeded during sepsis. However, DC activation also leads to the accumulation of innate immune cells [17]. In sepsis models, impaired monocyte metabolism causes remarkable immunosuppression characterized by an extensive inhibition of metabolic processes, such as glycolysis, fatty acid oxidation, and oxidative phosphorylation [18]. Furthermore, emerging paradigms indicate that proinflammatory and immunosuppression responses might occur simultaneously, and their intensity depends on multiple factors of both the host and the pathogen, such as genetics, comorbidities, and pathogen types [19].

#### **Mitochondrial dysfunction**

Due to the limited oxygen supply and incomplete oxidative reaction, free radical production dramatically increases while the machinery of the antioxidant system, such as mitochondrial homeostasis, becomes imbalanced during sepsis. Mitochondrial dysfunction can aggravate the failure of tissue oxygen extraction, which is called cytopathic hypoxia [20]. When exposed to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), activated leukocytes release inflammatory cytokines and subsequently trigger the expression of NADPH oxidase [21]. In addition, inflammatory cytokines induce overproduction of reactive nitrogen species (RNS) by activating inducible nitric oxide synthase (iNOS). NO can bind ROS peroxides to form RNS, resulting in the irreversible repression of electron transfer chain (ETC) activity. During sepsis, impaired mitochondria represent a source of additional ROS production, further contributing to damage to mitochondria, including mitochondrial DNA damage and intimal damage, which ultimately triggers the apoptosis process [22]. Mitochondrial biogenesis is regulated by the AMPK/PGC-1/NRF-1/2 signaling pathway. However, the activation of AMPK and the subsequent PGC-1/NRF-1/2 pathway is disrupted by the disproportionality of the ATP/ADP ratio, thus leading to a decreased mitochondrial density after a severe sepsis episode [23, 24].

#### **Coagulation disorders**

Sepsis usually interferes with the distribution of systemic blood flow to organ systems via vasodilation and disturbances in microcirculation [25]. Either a systemic or local mismatch between oxygen delivery and tissue demand can result in severe tissue ischemia. Inflammatory regulators are also involved in the coagulopathy underlying sepsis. The coagulation manifestation can vary from fibrin deposition to massive thromboembolism in the microvascular bed, leading to a severe complication termed disseminated intravascular coagulation [25]. When the maximal activation of coagulation occurs, endogenous fibrinolysis substantially diminishes in sepsis. The release of plasminogen activators, such as tissue plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), from vascular endothelial cell storage sites enhance the activity of plasminogen activators and the production of sub-quantitative plasmin [26]. Nevertheless, the disappearance of these effects is attributed to a continuous increase in plasminogen activator inhibitor-1 (PAI-1); a polymorphism of PAI-1 has been identified to increase the risk of septic shock caused by meningococcal infection [26].

#### **Neuroendocrine-immune network abnormalities**

The homeostasis involved in the interaction between the neuroendocrine and immune systems is also considered an important part of the host response during septic shock [27]. The responses of the central nervous system to sepsis depend on the following three main mechanisms: (1) circulatory inflammatory mediators connected to the central nervous system through the choroid plexus and ventricle organs; (2) the autonomic nervous system in which primary afferent nerves and sensory nerves are associated with PAMPs and enhance inflammatory cytokine activation; and (3) the activation of endothelial cells by the blood-brain barrier, leading to the release of inflammatory mediators, such as NOS metabolites [28]. Moreover, hypothalamic-pituitary-adrenal (HPA) axis dysfunction causes a reduction in the serum levels of corticotropin, adrenocorticotrophic hormone, and adrenal cortisol in sepsis, leading to adrenal insufficiency syndrome [29]. Evidence demonstrates that noradrenaline (NA) can respond to LPS by inhibiting the expression of proinflammatory factors, including TNF- $\beta$  and IL-12, and promoting the expression of anti-inflammatory cytokines, including IL-10 [30]. In addition, the concept of the "cholinergic anti-inflammatory pathway" has been proposed in a study examining how the vagus nerves participates in the regulation of sepsis, which opens up a new direction for the treatment of sepsis [31].

#### **Endoplasmic reticulum stress**

The endoplasmic reticulum (ER) is an intracellular organelle involved in protein translocation, folding, and posttranslational modification. Notably, unfolded or misfolded proteins are accumulated in the ER during sepsis, leading to impaired homeostasis and subsequent ER stress [32]. Under ER stress, unfolded protein response sensors might switch their signals to stimulate cell death by activating unique signaling mechanisms via multiple steps, including the transcriptional activation of the CEBP homologous protein (CHOP) gene and the activation of the JNK pathway [33]. In sepsis animal models, the increased expression of glucose-regulated protein 94, CHOP, and caspase-12, all of which are markers of enhanced ER stress, can be detected in sepsis-induced multiple organ failure, including heart and liver failure [33, 34]. In addition, ER stress results in abnormal apoptosis in sepsis animals, suggesting that ER stress-mediated apoptosis represents a potential novel target for the clinical prevention of sepsis [35].

#### **Autophagy**

Autophagy plays an essential role in not only resisting external pathogens and dangerous signals but also the regulation of the natural inflammatory immune-cell response during sepsis [36].

Autophagy-deficient mice lacking the autophagy protein or ATG16L1 gene are more susceptible to LPS challenge, and the endotoxin effects in these mice are enhanced due to the inhibited immune response as a result of T-cell autophagy deficiency [37]. Moreover, in a cecal ligation and puncture (CLP)-induced mouse model of sepsis, incomplete autophagy might lead to cardiac dysfunction in sepsis, which can be restored by autophagy activation using rapamycin [19]. Therefore, autophagy exerts a protective effect in sepsis via regulation of cytokine release, the reduction in the apoptosis process, and pathogen clearance.

### INFLAMMATORY STORM AND SEPSIS

The involvement of the inflammatory response in sepsis from the innate immune system consisting of macrophages, monocytes, neutrophils, and natural killer cells to PAMPs and DAMPs has been well described. PAMPs mainly include bacterial, fungal, and viral pathogens, such as endotoxin and  $\beta$ -glucan, while DAMPs mainly include endogenous molecules released from damaged host cells. Both PAMPs and DAMPs activate the immune cascade and several epithelial cells after the recognition of receptors on the cell surface (Toll-like receptors) or in the cytosol (NOD-like receptors), activating multiple cytokines, including IL-1, IL-6, and TNF- $\alpha$  [38, 39]. Inflammasomes assembled with some pattern recognition receptors are also essential for the secretion of several important cytokines, such as IL-1 $\beta$  and IL-18, resulting in inflammatory programmed cell death by pyroptosis [40]. In turn, proinflammatory cytokines enhance the number and activation state of innate immune cells and the expression of adhesion molecules and chemokines and promote neutrophils to release antimicrobial proteins and enzymes that form a scaffold for platelet activation [41]. In addition, tissue factor expression by blood monocytes and the release of NETs are upregulated by these increased inflammatory responses [42].

### BASIC BACKGROUND OF HMGB1

#### HMGB1

The human HMGB1 gene is located on chromosome 13 and is highly evolutionarily conserved [43]. HMGB1 is the most highly expressed of all HMG family members. The structure of HMGB1 is extremely conserved in eukaryotes among species, with 99% amino acid homology between rodents and humans [44]. HMGB1 binds DNA through its autologous structural domain to maintain the stability of the nucleosome and regulate transcription, translation, and DNA repair [45, 46]. Notably, the structural domains of HMGB1 perform different functions. Box B can bend and twine DNA, while Box A cannot [47, 48]. Blair and colleagues [49] discovered that the full-length (FL) HMGB1 bent DNA more than the individual A and B boxes. Removing the C-terminal tail of HMGB1 results in a protein that bends DNA to a greater extent than the FL protein. These data suggest that the A and B boxes simultaneously bind DNA in the absence of the C-terminal tail, but the tail modulates DNA binding and bending by one of the two HMG boxes in the FL protein.

HMGB1 is both a nuclear factor and a secreted protein. HMGB1 acts as an architectural chromatin-binding factor in the cell nucleus to bind DNA and promote protein assembly on specific DNA targets. Outside the cell, HMGB1 binds to receptor for advanced glycation endproducts (RAGE) with high affinity [50]. As a classic signaling molecule, HMGB1 plays diverse biological roles in pathological and pathophysiological processes, such as inflammation, immune reactions, cell migration, differentiation, proliferation, tissue repair, angiogenesis, aging, and cell death [51]. Interestingly, the functions of HMGB1 are determined by its autologous redox state. The reduced form of HMGB1 has been implicated in chemotaxis, whereas the disulfide form of HMGB1 is pro-inflammatory [52, 53]. Physiologically, the A box and B boxes

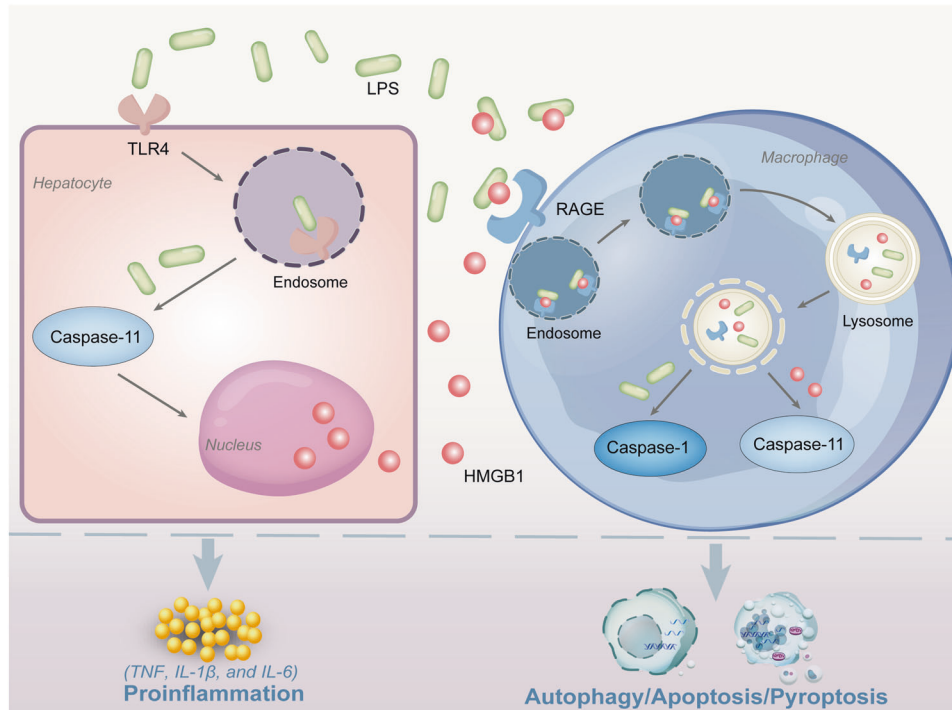
perform an internal action in the N-terminus called the reduced state, which promotes the recruitment of leukocytes and regeneration of tissues and organs [54]. However, the oxidation of HMGB1 results in the formation of intramolecular cysteine 23-cysteine 45 bonds, which enable HMGB1 to signal through TLR4-MD2 and function as a priming signal for NLRP3 inflammasome activation [52, 55]. Caspase activation targets mitochondria to produce reactive oxygen species (ROS), which are critical for the tolerance induction of apoptotic cells. ROS oxidize the potential danger signal HMGB1 released from dying cells and thereby neutralize its stimulatory activity. Apoptotic cells fail to induce tolerance and instead stimulate immune responses by scavenging or mutating target mitochondrial caspases when ROS activity is prohibited. Similarly, blocking oxidation sites in HMGB1 prevents tolerance induction by apoptotic cells [56].

#### Release of HMGB1

Physiologically, the levels of HMGB1 remain low in the cytoplasm and serum. However, the HMGB1 levels sharply increase under tissue injury [57]. HMGB1 is released from cells in the following two dependent ways: active secretion and passive release. Despite its clinical importance, the exact mechanism of HMGB1 release largely remains unknown. Extracellular HMGB1 performs its proinflammatory functions by binding immunostimulatory molecules, such as RNA, histone, lipopolysaccharide (LPS), and IL-1 $\alpha$  (Fig. 1) [58–60].

*Active secretion.* Evidence suggests that the HMGB1 protein is secreted by monocytes via a nonclassical, vesicle-mediated secretory pathway [61, 62]. Mechanistically, the activation of monocytes contributes to the redistribution of HMGB1 from the nucleus to cytoplasmic organelles that display the ultrastructural features of endolysosomes. Then, HMGB1 secretion is induced by stimuli triggering lysosomal exocytosis [63]. HMGB1 actively shuttles between the cytoplasm and nucleus, which is the first step of active secretion in almost all cells. Furthermore, the process of HMGB1 translocation from the nucleus to the cytoplasm significantly requires posttranslational modifications, such as phosphorylation and acetylation (Table 1). Nevertheless, the upstream pathway that phosphorylates HMGB1 is not fully understood [64]. Deng et al. [65] reported that calmodulin-dependent protein kinase (CaMK) IV may be involved in HMGB1 phosphorylation during the release of HMGB1 from LPS-stimulated macrophages. In monocytes and macrophages, HMGB1 is extensively acetylated by PCAF, CBP and p300 upon activation with LPS. In addition, increased hyperacetylation of HMGB1 in resting macrophages results in its relocalization to the cytosol. Then, cytosolic HMGB1 is concentrated by default in secretory lysosomes and subsequently secreted when monocytic cells receive an appropriate second signal [66]. More importantly, Deng et al. [67] recently found that hepatocytes rather than myeloid immune cells are the dominant source of systemic HMGB1 release during endotoxemia, which is dependent on the activation of caspase-11 to eventually induce gasdermin D-mediated pyroptosis of immune cells [68].

Moreover, interferon (IFN)- $\gamma$  induces HMGB1 release partially via a tumor necrosis factor (TNF)-dependent mechanism. Rendon-Mitchell and colleagues [69] discovered that IFN- $\gamma$ , which is an immunoregulatory cytokine known to mediate the innate immune response, dose-dependently induces the release of HMGB1, TNF, and nitric oxide. The pharmacological suppression of TNF with neutralizing antibodies or genetic knockout of TNF expression partially restrains IFN- $\gamma$ -mediated HMGB1 release. AG490, which is a specific inhibitor of Janus kinase (JAK) 2 of the IFN- $\gamma$  pathway, dose-dependently attenuates IFN- $\gamma$ -induced HMGB1 release. IFN- $\beta$ , IFN- $\gamma$ , and LPS [70–73], which are early proinflammatory cytokines, activate the JAK/signal transducer and activator of transcription (STAT) 1 pathway, which, in turn,



**Fig. 1 Release of HMGB1 and its intracellular and extracellular function in LPS-mediated infection and sepsis.** LPS uptake into hepatocytes via TLR4 can activate the caspase-11-mediated pathway to stimulate HMGB1 mobilization from the nucleus and release from hepatocytes. Extracellular HMGB1 can deliver LPS to macrophages. Subsequently, endosomal and lysosomal rupture in macrophages leads to the release of HMGB1 and LPS into the cell cytosol and activates caspase-11 and caspase-1 signaling. Consequently, HMGB1 induces a systemic inflammatory response through TNF, IL-1, and IL-6 and triggers cell apoptosis, autophagy, and pyroptosis. LPS lipopolysaccharide, HMGB1 high mobility group box 1, TLR4 Toll-like receptors.

**Table 1.** Modification and location-specific roles of HMGB1 in sepsis

Cell category	Modification	Effects	Intracellular or extracellular functions
Monocyte/macrophage	Phosphorylation	Translocation from nucleus to cytoplasm	Promote inflammation [64, 65]
Monocyte/macrophage	Acetylation	Translocation from nucleus to cytoplasm, delivery of LPS into the cytoplasm	Increase cytokines and chemokine production, induce autophagy and pyroptosis and inhibit macrophage cell death from endotoxemia [75]
Monocyte/macrophage	Reduced	Delivery of LPS into the cytoplasm	Promote chemotaxis [53]
Monocyte/macrophage	Disulfide	Promote inflammation response [54]	–
Hepatocyte	Phosphorylation	Translocation from nucleus to cytoplasm	Promote inflammation [79]
Hepatocyte	Acetylation	Translocation from nucleus to cytoplasm	Stabilize nucleus and mitochondria and induce inflammasome activation [67]
Hepatocyte	Reduced	Activate inflammasome [107]	–
Vascular endothelial cells	–	–	Increase inflammation response and interrupt endothelial integrity [55]
Platelet	–	–	Activate platelets in sepsis and thrombosis in trauma [76]

promotes the hyperacetylation of HMGB1. Then, HMGB1 is translocated from the nucleus to the cytoplasm and is subsequently released [74]. Notably, sirtuin1 (SIRT1) directly interacts with HMGB1 via its N-terminal lysine residues (28–30) and thereby suppresses HMGB1 release to improve survival in an experimental model of sepsis. In contrast, inflammatory stimuli, such as LPS and TNF- $\alpha$ , promote HMGB1 release by inducing its dissociation from SIRT1 to increase its acetylation, thereby increasing the association between HMGB1 and chromosome region maintenance 1, contributing to HMGB1 translocation [75]. In addition, platelet-derived HMGB1 is released and is involved in inflammation and thrombosis. Activated platelets release large amounts of HMGB1, which is transferred to the cytomembrane when blood vessels are injured [76, 77].

**Passive release.** HMGB1 can be released passively under a severe inflammatory response or lytic cell death, including necrosis, pyroptosis, and necroptosis, during sepsis and trauma. HMGB1 is loosely bound to chromatin in the interphase and mitotic cells and rapidly leaks into the medium when the membrane integrity is impaired in permeabilized or lytic cells [44]. HMGB1 knockout necrotic cells have a greatly reduced ability to promote inflammation, suggesting that the release of HMGB1 transmits the demise signal of a cell to adjacent cells. Apoptotic cells do not release HMGB1 even after undergoing secondary necrosis and partial autolysis and, thus, fail to promote inflammation, even if not cleared promptly by phagocytic cells. In apoptotic cells, HMGB1 is firmly bound to chromatin because of the generalized hyperacetylation of histone and is released into the extracellular

medium if chromatin deacetylation is prevented [50]. These findings are consistent with evidence suggesting that most HMGB1 released passively is non-acetylated HMGB1 still located within the nucleus [78]. Therefore, the non-acetylated form of HMGB1 has been utilized in investigations to clarify the proportion of HMGB1 passively released compared to its active release from cells in the inflammatory response. Moreover, HMGB1 has been observed to be in its absolute reductive form during passive release, which obviously affects the function and pro-inflammatory potential of HMGB1 [79]. Lipid peroxidation and aerobic glycolysis have been implicated in promoting HMGB1 release via inflammasome activation in sepsis [80, 81]. However, whether these metabolic stresses trigger the passive release of HMGB1 require further clarification. Altogether, the identification of the detailed mechanisms underlying the active and passive release of HMGB1 may provide optimal and effective therapeutics designed to suppress either lytic cell death or active HMGB1 release.

### HMGB1 AND THE INFLAMMATORY STORM

Disulfide HMGB1 has the most potent inflammatory effects and acts as a DAMP when released from cells in this form. HMGB1 can trigger massive inflammatory cytokine production from immune cells through multiple signaling pathways involving cell surface pattern recognition receptors, such as TLR4 and RAGE.

#### Toll-like receptors

Innate immune receptors for pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) orchestrate inflammatory responses to infection and sterile injury. Secreted by activated immunocytes or passively released from dying cells, HMGB1 is subjected to redox modification that distinctly influences its extracellular functions [55]. Toll-like receptors (TLRs) belong to an evolutionarily conserved type I transmembrane superfamily containing an extracellular leucine-rich repeat (LRR) and a cytoplasmic Toll/IL-1 receptor (TIR) domain [82–86]. Surface plasmon resonance studies indicate that HMGB1 specifically binds TLR4 and that this binding requires a cysteine at position 106. A wholly synthetic 20-mer peptide containing cysteine 106 within the cytokine-stimulating B box mediates TLR4-induced activation of macrophage TNF release. The inhibition of TLR4 binding with neutralizing anti-HMGB1 mAbs or by mutating cysteine 106 prevents the activation of HMGB1 [87]. In addition, Yang and colleagues [55] demonstrated that the extracellular TLR4 adaptor myeloid differentiation factor 2 (MD-2) specifically binds the cytokine-inducing disulfide isoform of HMGB1. These results suggest a requirement for HMGB1-dependent TLR4 signaling in MD-2-deficient mice and MD-2-silenced macrophages. P5779 (FSSE), which is a tetramer peptide for a specific MD-2 antagonist, prevents MD-2-HMGB1 interaction and TLR4 signaling. Mechanistically, P5779 does not interfere with LPS-induced cytokine/chemokine production, thus preserving PAMP-mediated TLR4–MD-2 responses. Furthermore, P5779 protects mice against sepsis. These findings reveal a novel mechanism by which innate systems selectively recognize specific HMGB1 isoforms [55]. Park and colleagues used fluorescence resonance energy transfer (FRET) and immunoprecipitation to directly investigate the cell surface interactions between HMGB1 and TLR2 or TLR4. Fluorescence resonance energy transfer images in RAW264.7 macrophages identified an association between HMGB1 and TLR2 or TLR4. Transient transfections of human embryonic kidney-293 cells showed that HMGB1 induced cellular activation and NF- $\kappa$ B-dependent transcription through TLR2 or TLR4. Coimmunoprecipitation also revealed an interaction between HMGB1 and TLR2 or TLR4. Moreover, during the release process of HMGB1 from hepatocytes, enhanced TLR4 signaling promoted both HMGB1

release from hepatocytes and LPS delivery to the cytosol of hepatocytes [67]. These studies provide the first direct evidence suggesting that HMGB1 interacts with both TLR2 and TLR4 to activate inflammatory responses [88].

#### RAGE

RAGE was first described as a signal transduction receptor for advanced glycation endproducts (AGE) [89–91]. Kokkola and colleagues [92] found that macrophages from RAGE<sup>-/-</sup> mice produced significantly lower levels of TNF, IL-1 $\beta$ , and IL-6 in response to HMGB1 stimulation. Under physiological conditions, RAGE mediates interactions with ligands that are distinct from AGE. Cultured embryonic rat neurons, which express RAGE, dose-dependently bind <sup>125</sup>I-amphotericin, which is restrained by blocking RAGE using antibodies against the receptor or excess soluble receptor (sRAGE) [93]. Recent evidence has revealed that HMGB1 is sensitive to cleavage by caspase-1 activation, resulting in the production of a fragment within its N-terminal DNA binding domain (A-box) that reverses apoptosis-induced tolerance by binding RAGE. In a double-hit mouse model of sepsis initially injected intraperitoneally with a low dose of LPS (400 mg/kg) for 6 h and subsequently challenged with a high dose of LPS (10 mg/kg), LeBlanc et al. [94] discovered that tolerance to secondary infection and its associated mortality were effectively reversed by the active immunization of dendritic cells treated with HMGB1 or the A-box fragment but not a form of HMGB1 that cannot be cleaved. In addition, HMGB1 could initiate endocytosis and induce a cascade of molecular events, including cathepsin B release from ruptured lysosomes, which triggers caspase-1 activation and macrophage pyroptosis through RAGE-dependent signaling. Furthermore, the pyroptosis induced by HMGB1 has also been observed in vivo during endotoxemia, highlighting the pathophysiological significance of HMGB1 in pyroptosis during the development of inflammation [95]. Notably, Deng et al. [67] showed that HMGB1 could significantly mediate caspase-11-dependent pyroptosis and lethality in sepsis by delivering extracellular LPS to the cytosol of macrophages and endothelial cells [96]. The important mode in this process is the RAGE-mediated internalization of HMGB1-LPS complexes. Taken together, these findings represent a novel link between RAGE and HMGB1 delivery and release with potential therapeutic implications for inflammatory diseases (Fig. 1).

### ROLES OF HMGB1 IN SEPSIS AND SEPSIS-RELATED ORGAN INJURY

#### Sepsis-related lung injury

Acute lung injury (ALI) is closely correlated with sepsis in the intensive care unit setting [97–100]. Sepsis-induced ALI has been reported to have a higher fatality rate than other pathogens that trigger ALI [101]. Lan and colleagues [102] reported that salidroside ameliorates sepsis-induced ALI and mortality in mice by downregulating the SIRT1/HMGB1 pathway. Salidroside, which is a substance isolated from *Rhodiola rosea*, possesses antioxidant and anti-inflammatory properties. Salidroside suppresses the inflammatory responses and HMGB1 production and reverses the reduced expression of the SIRT1 protein in bacterial LPS-treated macrophages and mice. Moreover, salidroside alleviates sepsis-induced lipid peroxidation, histopathological changes and mortality in a lung edema model and improves the lung PaO<sub>2</sub>/FiO<sub>2</sub> ratio in CLP-induced septic mice. Sepsis decreases SIRT1 protein expression in the lungs of CLP-induced septic mice. However, salidroside significantly upregulates SIRT1 expression and inhibits inflammatory responses in CLP-induced septic mouse lungs [102]. The injection of Xuebijing, which is a traditional Chinese medicine, has been reported to be a promising approach in the treatment of sepsis in China. Xuebijing reduces morphological destruction,

neutrophil infiltration and the lung wet/dry weight ratio in the alveolar space, which improves mortality in cecal ligation and puncture (CLP)-induced lung injury. Moreover, Xuebijing treatment downregulates HMGB1 and RAGE expression, neutrophil counts and production of IL-1, IL-6, and TNF- $\alpha$  in bronchoalveolar lavage fluid. These results indicate that Xuebijing reduces mortality in mice with CLP-induced lung injury during sepsis by inhibiting the proinflammatory cytokine secretion mediated by the HMGB1/RAGE axis [103]. The rats in the CLP + ulinastatin group exhibited higher 7-d survival rates, less lung injury, and reduced HMGB1 expression in the lung tissue, serum, and bronchoalveolar lavage fluid (BALF) compared with those in the CLP group. In addition, the levels of TNF- $\alpha$  and IL-6 at 24 h in the CLP + ulinastatin group were markedly lower than those in the CLP group. These results suggest that ulinastatin decreases lung injury and increases the survival time of ALI rats by downregulating HMGB1 expression and inhibiting TNF- $\alpha$  and IL-6 levels in serum and bronchoalveolar lavage fluid [104].

CLP increases the wet/dry weight ratio and myeloperoxidase activity in the lung by upregulating HMGB1 and enhancing the number of inflammatory cells, such as neutrophils and macrophages, in the BALF. Moreover, the expression of HMGB1 and RAGE in lung tissues is increased after CLP, whereas ketamine reverses all of the above effects. Ketamine also suppresses the activation of I $\kappa$ B- $\alpha$ , NF- $\kappa$ B, p65, and mitogen-activated protein kinases (MAPK). This finding reveals that ketamine protects rats against HMGB1-RAGE activation in a rat model of sepsis-induced ALI [105]. In a mouse model of ALI, blocking HMGB1 or a myeloid-specific PTEN knockout (PTENM-KO) increases animal survival and body weight, reduces the expression of IL-17 and lung damage, increases TGF- $\beta$  production, promotes  $\beta$ -catenin signaling, and subsequently induces CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs to suppress endotoxin-mediated inflammation in LPS- or rHMGB-induced lung injury. Notably, myeloid-specific  $\beta$ -catenin ablation ( $\beta$ -catenin M-KO) results in reduced animal survival and increased lung injury in rHMGB-induced ALI. Furthermore, the disruption of macrophage HMGB1/PTEN or activation of  $\beta$ -catenin significantly increases CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Tregs, contributing to repressed endotoxin-induced inflammation responses in vitro. These findings suggest that HMGB1/PTEN/ $\beta$ -catenin signaling is a novel pathway that regulates Treg development and provides a potential therapeutic target for sepsis-induced lung injury [106].

#### Other sepsis-related injuries

HMGB1 also plays a pivotal role in other sepsis-related organ injuries and diseases. In sepsis-induced liver injury models, sepsis-related serum aminotransferase activity, and proinflammatory chemokine levels are reduced by resveratrol pretreatment, which also improves liver histological parameters that are complicated by SIRT1 upregulation and HMGB1 downregulation. Mechanistically, resveratrol suppresses the cytoplasmic translocation of HMGB1 and decreases the inflammatory responses in hepatocytes [107, 108]. In addition, Zheng and colleagues reported that the mRNA transcription levels of IL-1 and IL-6 in renal tubular epithelial cells (RTECs) were significantly increased during sepsis, accompanied by accumulated HMGB1 in renal tissue that entered urine during sepsis. These authors further found that HMGB1 turns RTECs into inflammatory promoters by interacting with TLR4 during sepsis [109]. Bacterial sepsis not only triggers innate immune responses but also extensively stimulates coagulation cascades that leads to disseminated intravascular coagulation (DIC). Type 1 IFNs significantly mediate bacterial infection-induced DIC by enhancing the release of HMGB1 into the blood stream, where it binds and delivers LPS into the cytoplasm of the host cell, consequently activating caspase-11-dependent immune responses and dysfunction of endothelial cells [110].

#### POTENTIAL THERAPEUTICS

Recently, several novel interventions targeting HMGB1 for sepsis treatment have emerged. Glycyrrhizin (GL), which is also known as glycyrrhizic acid, is a natural triterpene glycoside and the major active component of Gancao. Zhao and colleagues discovered that GL significantly reduced the serum levels of HMGB1 in vivo. GL also ameliorates the release and expression of HMGB1 and HMGB1-related proinflammatory cytokines. Furthermore, GL blocks the interaction between HMGB1 and RAGE or TLR4 and suppresses the downstream MAPKs/NF- $\kappa$ B pathway [111]. Naringin, which is a flavanone glycoside extracted from various plants, has a wide range of pharmacological effects. Naringin increases the expression of HMGB1 and heme oxygenase (HO)-1 in peritoneal macrophages by activating adenosine monophosphate-activated protein kinase, p38, and NF-E2-related factor 2 (Nrf2). Mechanistically, naringin restrains HMGB1 release from LPS-stimulated macrophages and improves survival in CLP-induced septic mice [112]. However, the appropriate dose and intervention time for the use of these inhibitors, such as GL and Naringin, in sepsis are unclear and require further investigation. Moreover, many reports regarding the successful utilization of polyclonal or mouse anti-HMGB1 monoclonal antibodies in experimental inflammatory models have been published. To date, different humanized anti-HMGB1 monoclonal antibodies and HMGB1-neutralizing Affibody molecules have also been identified in many preclinical studies investigating inflammatory conditions. As investigations aiming to identify the conditions under which the concentrations of HMGB1 are suppressed are still ongoing, future clinical investigations focusing on HMGB1 inhibitors may focus on (1) exploring the safety and efficacy of the preventive use of HMGB1 in the clinic; (2) identifying the more accurate mode of inhibitory action and verifying the bioactivity of a given batch of inhibitors; (3) determining how to maintain HMGB1 at appropriate levels to prevent damage without inhibiting tissue regeneration and wound healing as a balanced therapeutic mechanism; and (4) confirming whether the clinical application of therapeutics targeting HMGB1 has other unsatisfactory side effects.

Although abundant preclinical evidence regarding the efficacy of targeting HMGB1 in inflammation is available, a detailed mechanistic understanding of the structure-function interactions between HMGB1 and inflammatory receptors or signaling pathways remains lacking. Studies may provide more preclinical evidence for clinical translation that should include evaluations of (1) whether the upregulation or disparate intracellular location of HMGB1 is a cause or a consequence of the progression of sepsis; (2) which type(s) of post-transcriptional or post-translational regulation contributes to HMGB1 upregulation or activation in sepsis; and (3) the identification of more downstream targets or pathways activated by HMGB1 in mitochondrial dysfunction and inflammation storms.

Surprisingly, HMGB1 is regarded as an essential predictor of organ dysfunction and outcome in patients with severe sepsis. Van and colleagues [113] revealed that the HMGB1 levels were increased in severe sepsis patients but were not correlated with the outcome. Moreover, another investigation showed that there was no difference in the HMGB1 concentrations between patients with septic shock and patients with severe sepsis [114]. Recently, Karlsson et al. [115] enrolled 247 adult patients with severe sepsis. The serum HMGB1 concentrations were elevated in the patients with severe sepsis after 72 h but did not differ between the survivors and nonsurvivors and did not predict hospital mortality. Thus, more work is still needed to elucidate the clinical significance of HMGB1 in sepsis.

Intriguingly, under normal physiological conditions, the serum levels of HMGB1 are attenuated in humans with aging, which contrasts the detrimental effects of increased HMGB1 under pathologic conditions [116]. In many aging studies, the HMGB1

plasma levels were found to be associated with organ failure [117, 118]. Additionally, immunosenescence alters the role of sepsis as a cause of death in the elderly [6]. Here, the inhibition of HMGB1 is pursued as a therapeutic target in sepsis. HMGB1 acts as a late mediator of inflammation and contributes to prolonged and sustained systemic inflammation in a model of aging-related osteoarthritis [119]. Interestingly, studies involving the senescence inhibitor metformin revealed that its direct binding to HMGB1 suppresses the pro-inflammatory activity of HMGB1 [120].

## CONCLUSION

Sepsis remains a common and deadly problem worldwide. The discovery of HMGB1 as a potent cytokine mediator of sepsis has sparked a new field of investigation related to the treatment of sepsis. HMGB1 plays important roles as a DAMP mediator of inflammation involved with TLR and RAGE pathways in the pathogenesis of sepsis. The cell-type specific targeting of inhibitors of HMGB1 will likely be necessary. Here, we propose that the inhibition of HMGB1 might be a potential therapeutic avenue against sepsis and sepsis-related organ injury. Although several lines of evidence have clarified the underlying mechanisms of HMGB1 in sepsis, the development of a successful treatment that includes HMGB1 has not been instituted to date. Further investigations may focus on (1) identifying the relationship between HMGB1 concentrations and the progression of sepsis; (2) determining the unknown mechanisms by which HMGB1 regulates sepsis; and (3) performing a high-quality systematic review or a meta-analysis to provide evidence-based support. Overall, this review presents a detailed view of HMGB1 in sepsis and indicates the potential of HMGB1 as a therapeutic target for sepsis-related organ injury.

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## ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

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