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REVIEW

Role of High Mobility Group Box 1 in Inflammatory Disease: Focus on Sepsis

Jong-Sup Bae
College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 702-701, Korea

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High mobility group box 1 (HMGB1) is a highly conserved, ubiquitous protein present in the nuclei and cytoplasm of nearly all cell types. In response to infection or injury, HMGB1 is actively secreted by innate immune cells and/or released passively by injured or damaged cells. Thus, serum and tissue levels of HMGB1 are elevated during infection, and especially during sepsis. Sepsis is a systemic inflammatory response to disease and the most severe complication of infections, and HMGB1 acts as a potent proinflammatory cytokine and is involved in delayed endotoxin lethality and sepsis. Furthermore, the targeting of HMGB1 with antibodies or specific antagonists has been found to have protective effects in established preclinical inflammatory disease models, including models of lethal endotoxemia and sepsis. In the present study, emerging evidence supporting the notion that extracellular HMGB1 acts as a proinflammatory danger signal is reviewed, and the potential therapeutic effects of a wide array of HMGB1 inhibitors agents in sepsis and ischemic injury are discussed.

Key words: HMGB1, LPS, Sepsis, Infection, HMGB1 inhibitor

INTRODUCTION

The immune system has both innate (inherited) and adaptive (acquired) components. Innate immunity is an inherited type of immune protection, whereas adaptive immunity develops throughout life (Brightbill et al., 1999). Cells of the innate immune system, such as, monocytes, macrophages, and neutrophils, represent the front line of host response to infection, invasion, and injury (Wang et al., 2008). During infection, innate immune cells recognize pathogen-associated molecular patterns (PAMPs), such as, lipopolysaccharide (LPS), bacterial peptidoglycan, double stranded RNA, CpG DNA, and enterotoxins, or damage-associated molecular pattern (DAMPs), such as, heat shock proteins, uric acid, annexins, interleukin (IL)-1α, and high mobility group box 1 (HMGB1), using recognition receptors (e.g., the Toll-like receptors, TLR 2, 4, and 9) (Brightbill et al., 1999; Wang et al., 1999a; Li et al., 2003). Subsequently, innate immune cells infiltrate infected tissues (Luster et al., 2005), and release various cytokines like tumor necrosis factor (TNF), IL-1, IL-6, and IL-12 and chemokines like IL-8, macrophage inflammatory protein (MIP)-1s, MIP-2, and monocyte chemotactic protein (MCP-1) (Baggiolini and Loetscher, 2000; Wang et al., 2008). In response to microbial infection or injury, the innate immune system mounts an immediate biological response, that is, inflammation, to remove invading pathogens (Wang et al., 2008). After pathogens have been eliminated, inflammation normally subsides to restore immunologic homeostasis (Serhan and Savill, 2005). On the other hand, exogenous pathogens or endogenous proinflammatory mediators can leak into the bloodstream and trigger a systemic inflammatory response that may lead to severe vascular inflammatory disease, that is, sepsis (Wang et al., 2008) (Fig. 1). Sepsis describes a systemic inflammatory response syndrome resulting from a microbial infection, and it terms of its clinical severity, sepsis can progress to severe sepsis or septic shock (Dellinger et al., 2008). Here, I present a brief review of the prevailing theories of sepsis and discuss potential therapeutic agents that target a late mediator of experimental sepsis.

Correspondence to: College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 702-701, Korea
Tel: 82-53-950-8570, Fax: 82-53-950-8557
E-mail: baejs@knu.ac.kr

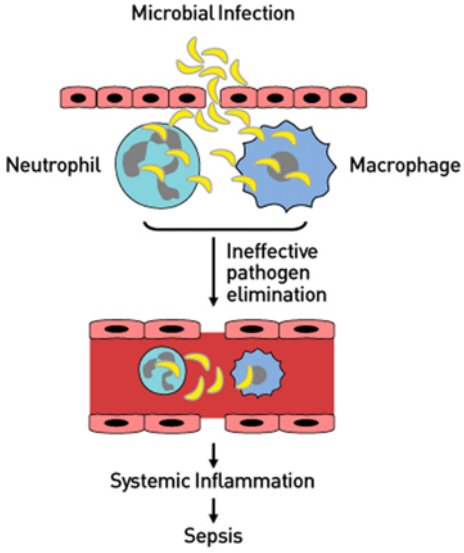

Fig. 1. Microbial infections can trigger local or systemic inflammatory responses. Upon disruption of the endothelial barrier, microbial pathogens invade and elicit an innate immune response at the infection site. If invading pathogens are ineffectively eliminated, they can leak into the blood stream and trigger a potentially injurious systemic inflammatory response and subsequent sepsis.

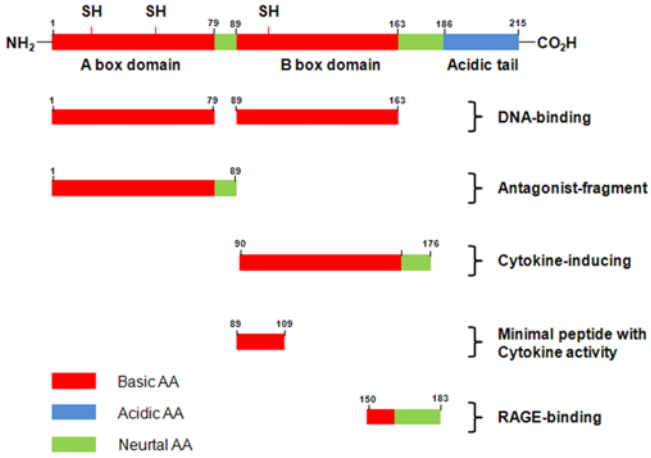

Fig. 2. Domain structure of HMGB1. HMGB1 is a conserved chromosomal protein composed of two similar DNA binding domains (A and B box) linked by a short basic stretch to an acidic C-terminal tail of 30 residues. Green shades are neutral amino acids; Red shaded amino acids are basic and blue shaded amino acids are acidic residues.

HMGB1 in inflammation

HMGB1 is highly conserved through evolution, and has 99% identity among all mammals. Out of its 215 amino acids, only two residues are substituted in the rodent and human versions. HMGB1 has two DNA-binding domains – the A-box (aa 1-79) and the B-box (aa 89-163) – and a highly acidic, repetitive C-terminal tail (aa 186-215) (Fig. 2) (Paonessa et al., 1987; Wen et al., 1989; Ferrari et al., 1996). Several proinflammatory activities of HMGB1 have been revealed from *in vitro* and *in vivo*. A picture has emerged of HMGB1, released into the extracellular milieu, having significantly pro-inflammatory functionality. HMGB1 is also a potent pro-inflammatory cytokine and associates with a variety of inflammatory diseases, especially sepsis

Receptors mediating HMGB1 activity

Once released into the extracellular milieu, HMGB1 can bind to cell surface receptors including the RAGE, TLR2, TLR4, and TLR9 (Wang et al., 2001, 2009; Yang et al., 2005). HMGB1 interaction with these receptors transduces intracellular signals and mediates cellular responses including chemotactic cell movement and release of pro-inflammatory cytokines.

RAGE

HMGB1 binds to RAGE in a concentration-dependent manner (Hori et al., 1995). As a receptor of multiple ligands, RAGE has also been implicated as a receptor mediating the chemotaxis and cytokine activity of HMGB1 in macrophages and tumor cells (Degryse et al., 2001; Yang et al., 2005; Wang et al., 2009). A structure/functional analysis revealed that amino acids 150-183 in the C terminus of HMGB1 are responsible for RAGE binding (Huttunen et al., 2002). Anti-RAGE antibodies partially inhibit HMGB1-induced chemokine and cytokine release in endothelial cells (Fiuza et al., 2003). Hence, HMGB1 interacts with RAGE, but interaction with TLR2 and TLR4 are required for HMGB1 signaling in macrophages.

TLR4

Toll-like receptors (TLRs) are highly conserved proteins that activate innate immune cells in response to a variety of endogenous and exogenous stimuli. Two of the TLRs have been reported to be involved in HMGB1 signaling: TLR2 and TLR4. TLR4 is suggested as the primary receptor in mediating macrophage activation, cytokine release and tissue injury (Tsung et al., 2005, 2007a, 2007b). HMGB1 signals via TLR4 in human whole blood, and primary macrophages to induce cytokine release. HMGB1-stimulated TNF release is inhibited in macrophages obtained from MyD88 (a transducer for TLR protein signaling) or TLR4 knock out mice, but not from TLR2 knock out mice or wild type controls (Yu et al., 2006; Apetoh et al., 2007). HMGB1 signals via TLR4 to activate tumor antigenspecific T cell immunity in both mice and humans (Apetoh et al., 2007). *In vivo* studies of ischemia-reperfusion suggest a role of TLR4 in HMGB1 mediated tissue injury. *In vitro*, HMGB1

directs inflammatory responses mediated by dendritic cells in ischemia-reperfusion models by enhancing TLR4 expression (Tsung et al., 2007b).

TLR2

HMGB1 can also elicit cellular signaling through TLR2. Using dominant negative constructs to block MyD 88, TLR2 or TLR4 genes in macrophages *in vitro*, Park et al. observed that TLR2 and TLR4 are involved in cellular activation by HMGB1 (Park et al., 2004). In human embryonic kidney (HEK293) cells transfected with TLR2, TLR4, or vector alone, HMGB1 effectively induces IL-8 release only fromTLR2 over-expressing cells. Consistently, anti-TLR2 antibodies dose-dependently attenuate HMGB1-induced IL-8 release in HEK/TLR2-expressing cells and markedly reduce HMGB1 cell surface binding on murine macrophage-like RAW 264.7 cells (Yu et al., 2006). Fluorescent resonance energy transfer (FRET) and immuno-precipitation analyses in macrophages showed that HMGB1 binds to TLR2 and TLR4 on cell surface, but not RAGE (Park et al., 2006).

HMGB1 mediation of inflammatory response *in vitro*

HMGB1 can activate inflammatory responses in various cell types under *in vitro* conditions. For instance, recombinant HMGB1 dose-dependently upregulates TNF mRNA and protein expression in human primary blood mononuclear cell cultures, with an early peak appearing 4 h after addition of rHMGB1, and a subsequent peak beginning at 10 h post stimulation (Andersson et al., 2000). This biphasic TNF response to HMGB1 is distinct from the monophasic, rapid kinetics of TNF typically observed after endotoxin stimulation (Andersson et al., 2000). Similarly, in response to HMGB1 stimulation, human microvascular endothelial cells increase expression of adhesion molecules (such as ICAM-1 and VCAM-1), as well as secretion of proinflammatory cytokines including TNF and IL-8 (Fiuza et al., 2003), suggesting that HMGB1 can propagate an inflammatory response in the endothelium during infection or injury. In neutrophils, HMGB1 activates MAPKs (such as p38 and ERK1/2), phosphatidylinositol 3-kinase/Akt, and enhances the expression of proinflammatory cytokines in NF-κB-dependent fashion (Park et al., 2003), indicating an important role of HMGB1 in the activation of neutrophils during infection and injury. HMGB 1 has two conserved DNA-binding domains, referred to as HMGB1 boxes 'A' and 'B' (Li et al., 2003). Structure/function analyses revealed that B box recapitulate the cytokine activity of full length HMGB1, and efficiently activate macrophages to release TNF and other proinflammatory cytokines (Li et al., 2003). The HMGB1 B box thus conveys dual functionality to HMGB1, both as a cytokine and a DNA binding protein (Li et al., 2003). Exposure of epithelial cell monolayers to HMGB1 or B box increased the permeability of Caco-2 monolayers to fluorescein isothiocyanate-labeled dextran (FD4) in a time- and dose-dependent fashion, which implicated HMGB1 as a mediator of epithelial barrier dysfunction (Sappington et al., 2002). Epithelial leakage mediated by HMGB1 requires signaling through MAP kinases, NF-κB, and nitric oxide (Sappington et al., 2002) suggesting HMGB1 B box induces functional alterations in the epithelia of the gut or other organs that may contribute to the pathogenesis of lethal systemic inflammation. Neutrophilic inflammatory response show no significant difference in mice which were i.p. challenged with necrotic Hmgb1+/+ and Hmgb1-/- cells (Chen et al., 2007) suggesting that HMGB1 may augment the inflammatory response by binding to bacterial product.

HMGB1 mediation of inflammatory response in animal model study

Intratracheal administration of HMGB1 to LPS resistant mice stimulates lung neutrophil accumulation, and local production of proinflammatory cytokines, including IL-1β, TNF and MIP-2 in the lung tissue (Fig. 3) (Abraham et al., 2000a). Histological examination of lung tissue reveals evidence of an acute diffuse inflammatory response, with accumulation of neutrophils in the interstitial and intra-alveolar areas, interstitial edema, and protein exudation into the alveolar space

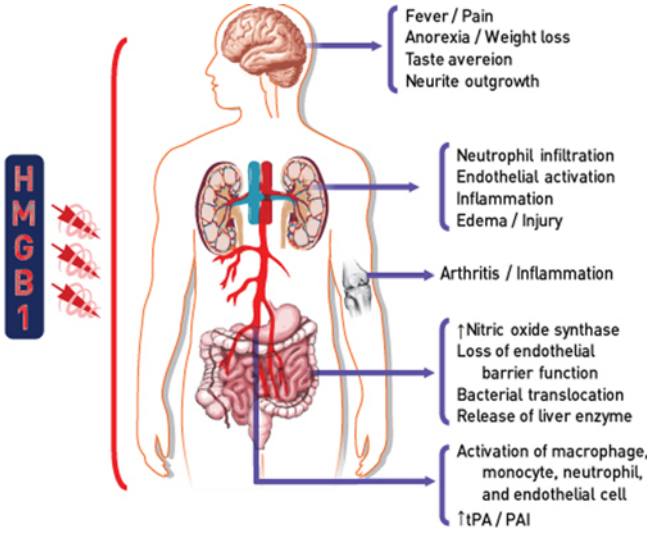

Fig. 3. HMGB1-mediated proinflammatory responses at various sites. Administration of HMGB1 via intracerebroventricular, intratracheal, intraperitoneal and intraarticular routes induces marked inflammatory responses, and activate various innate immune cells.

(Abraham et al., 2000a). Intracerebroventricular administration of HMGB1 increases brain TNF and IL-6 production, and induces sickness behaviours including anorexia and taste aversion (Agnello et al., 2002). Passive immunization with anti-HMGB1 antibodies attenuates the development of LPS-induced hypophagia, indicating that HMGB1 is a mediator of sickness behaviour associated with endotoxaemia (Agnello et al., 2002). Focal administration of HMGB1 in the region of the sciatic nerve induces dose dependent unilateral and bilateral low threshold mechanical allodynia (Chacur et al., 2001), suggesting a role in causing pathological pain during inflammation. Administration of HMGB1 B box to mice increases both ileal mucosal permeability and bacterial translocation to mesenteric lymph nodes (Fig. 3) (Sappington et al., 2002). A box also can neutralize the cytokine activity of HMGB1 in sepsis and protects against endotoxin and sepsis lethality, furthermore specific inhibition of endogenous HMGB1 such as anti-HMGB1 Ab or RAGE antagonists could reverse the lethality of established sepsis by preventing the development of organ damage in a model of murine (Yang et al., 2004; Qin et al., 2006). Proinflammatory cytokines stimulate release of HMGB1 from pituicytes and astrocytes (Passalacqua et al., 1998; Wang et al., 1999b) suggesting that HMGB1 involve in neurological disorders. It also demonstrates that HMGB1 is highly expressed in mouse brain neurons and astrocytes, and released during brain ischemia and contributes to neuroinflammation and post-ischemic brain damage (Faraco et al., 2007). Considered together, these studies indicate that accumulation of HMGB1 can amplify the cytokine cascade, stimulate inflammation, and mediate injurious inflammatory responses.

Clinical studies

HMGB1 is secreted by activated monocytes/macrophages, and is passively released by necrotic or damaged cells (Scaffidi et al., 2002). Clinical observational studies have demonstrated HMGB1 is a late mediator of sepsis in amplifying the inflammatory response that follows acute tissue damage pointed to this protein as a member of this group of "danger molecules", and investigations have reported elevated serum/plasma HMGB1 concentrations in patients with sepsis (Wang et al., 1999a; Ueno et al., 2004). Septic patients who succumbed to infection had higher serum HMGB1 levels than those that survived, suggesting that this protein warrants investigation as a therapeutic target (Wang et al., 1999a). Previously, high HMGB1 concentrations were found at the site of local tissue infection. Ueno found high HMGB1 levels are present in the pulmonary epithelial lining fluid of patients with sepsis (Ueno et al., 2004). Serum of HMGB1 levels increase in sepsis despite the primary source of infection, although in urinary tract infection, HMGB1 secretion into the circulation may be delayed when compared with pneumonia and peritonitis (van Zoelen et al., 2007). Other studies demonstrate that HMGB1 can trigger inflammation and also functions as a late mediator of endotoxemia and sepsis in patients (Hatada et al., 2005; Sunden-Cullberg et al., 2005). van Zoelen also found HMGB1 release may predominantly occur at the site of infection in patients with severe infection (van Zoelen et al., 2007). Kornblit et al. report that the genetic variation in the HMGB1 gene could affect the outcome in patients with systemic inflammatory response syndrome (SIRS) and sepsis, suggesting a possible role for HMGB1 genetics in future (Kornblit et al., 2008).

Release of HMGB1

To act as an effective danger signal and inflammatory mediator, HMGB1 must be transported extracellularly. This occurs in two different ways, namely, by its active secretion from living inflammatory cells and by it passive release from necrotic cells.

Active secretion of HMGB1

Intriguingly, HMGB1 lacks a classic leader peptide and does not travel through the endoplasmic reticulum or Golgi apparatus. Nevertheless, large amounts of HMGB1 are released into the extracellular space by activated monocytes and macrophages (Andersson and Tracey, 2011). In a previous report, it was suggested that the secretion of HMGB1 requires at least three steps: (i) exit from the nucleus into the cytoplasm, (ii) translocation from the cytosol into cytoplasmic organelles, and (iii) exocytosis (Andersson and Tracey, 2011). LPS and TNF-α stimulate HMGB1 secretion from monocytes/macrophages via different pathways. LPS stimulates macrophages to release HMGB1 by hyperacetylation partly via a CD14- and TNF-dependent pathway and via an IFN-β-mediated JAK/STAT pathway (Andersson and Tracey, 2011). However, TNF-α stimulates macrophages to secrete HMGB1 through phosphorylation (Youn and Shin, 2006). The majority of HMGB1 secreted by activated monocyte and macrophages is from a preformed cellular pool within 16 h of insult, and subsequently, increased cellular synthesis of HMGB1 further reinforces HMGB1 release into the extracellular milieu (Wang et al., 1999a).

Passive release of HMGB1

The passive release of HMGB1 from necrotic cells was demonstrated in 2001 (Li et al., 2001). In this study, cells rendered necrotic by repeated freeze thawing were

found to release their nuclear HMGB1 into supernatant, whereas apoptotic cells retained their HMGB1 bound to DNA. In contrast, necrotic *hmgb1*-gene deficient fibroblasts induced only low levels of TNF in macrophage cultures, thus indicating HMGB1 is a major factor in necrosis-induced inflammation and that it fulfills the criteria of an endogenous danger signal. The mechanism responsible for the retention of HMGB1 in the nuclei of apoptotic cells was found to involve the underacetylation of histones during the apoptotic process (Li et al., 2001). Furthermore, the HMGB1 passively released by necrotic cells and the HMGB1 actively secreted by macrophages are molecularly different, that is, actively secreted HMGB1 is acetylated (Andersson and Tracey, 2011).

Extracellular HMGB1 as a danger signal

HMGB1 was originally described as a nuclear protein that binds DNA, and functions as a co-factor that is required for transcriptional regulation and gene expression (Wang et al., 1999a). Furthermore, HMGB1 is a potent proinflammatory cytokine and has been associated with a variety of inflammatory diseases, especially sepsis (Andersson and Tracey, 2011), and thus, has been categorized as a danger signal (Fig. 4).

Definitions of sepsis and septic shock

Table I provides definitions for bacteraemia, sepsis, severe sepsis, septic shock and multiple organ dysfunction syndrome (MODS) (Abraham et al., 2000b). Systemic inflammatory response syndrome (SIRS) can be precipitated by a variety of underlying conditions even in the absence of infection (Abraham et al., 2000b). Sepsis is a systemic inflammatory response to infection.

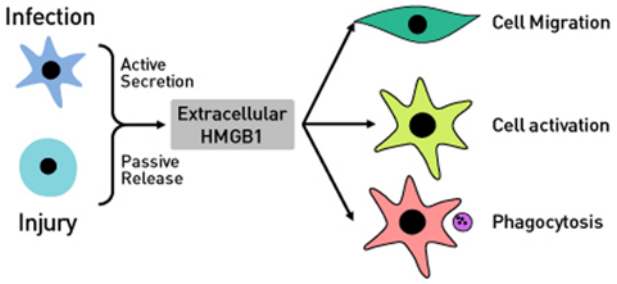

Fig. 4. Microbial infections can trigger local or systemic inflammatory responses. Extracellular HMGB1 functions as a danger signal. HMGB1 is actively secreted by innate immune cells in response to exogenous microbial products or endogenous host stimuli and passively released by damaged or virus-infected cells. Furthermore, extracellular HMGB1 sustains inflammatory response by stimulating the migration of innate immune cells, facilitating the recognition of bacterial products by the innate immune system, activating various innate immune cells, and by suppressing the phagocytosis of apoptotic cells. Thus, HMGB1 can function as a danger signal to recruit, alert, and activate various innate immune cells, and thereby, sustain inflammatory response.

Severe sepsis is sepsis with evidence of organ dysfunction such as hypoxaemia, oliguria, lactic acidosis or altered cerebral function (Abraham et al., 2000b). Septic shock is severe sepsis complicated by hypotension defined as systolic blood pressure less than 90 mmHg despite adequate fluid resuscitation. The process begins with an infection, with or without a systemic inflammatory response, and may progress to a systemic response with severe sepsis or septic shock (Abraham et al., 2000b). Sepsis and SIRS may be complicated by the failure of two or more organs, termed multiple organ failure (MOF), due to suboptimal organ perfusion and

Table I. Definitions for systemic inflammatory response syndrome and sepsis

Terms	Definitions
SIRS	The widespread inflammation that occurs following a wide variety of insults (including infection, pancreatitis, trauma, burns, etc.) regardless of the presence of infection
Bacteremia	Presence of viable bacteria in the circulating blood confirmed by culture
Sepsis	SIRS due to infection. The systemic response of sepsis includes two or more abnormalities of 1. Temperature > 38°C or < 36°C 2. Tachycardia > 90 beats/min 3. Respiratory rate > 20 breaths/min or $PaCO_2$ < 4.3 kPa 4. White blood count >12 × 10^9/L or < 4 × 10^9/L or > 10% immature (band) forms
Severe sepsis	Sepsis with evidence of organ hypoperfusion, made evident by signs of organ dysfunction such as hypoxaemia, oliguria, lactic acidosis or altered cerebral function
Septic shock	Severe sepsis with hypotension (systolic blood pressure < 90 mmHg) despite adequate fluid resuscitation or the requirement for vasopressors/inotropes to maintain blood pressure
MOF	Failure of two or more organs due to disordered organ perfusion and oxygenation

SIRS, systemic inflammatory response syndrome; MOF, multiple organ failure.

oxygenation (Abraham et al., 2000b).

The stimulation of cell migration by HMGB1

Accumulating evidence indicates that HMGB1 can stimulate the migrations of various cell types, including, neurites, smooth muscle cells, tumor cells, mesoangioblast stem cells, monocytes, dendritic cells, and neutrophils (Andersson and Tracey, 2011; Bae and Rezaie, 2011) (Fig. 4), which raises the possibility that extracellular HMGB1 might facilitate the recruitment of innate immune cells to sites of infection or injury, and thereby, function as a potential host cell derived chemotactic factor (Andersson and Tracey, 2011).

Facilitation of the innate recognition of microbial products by HMGB1

Recent studies suggest that HMGB1 can binding to and facilitate the innate recognition of bacterial products (e.g., CpG-DNA or LPS) by innate immune cells, such as, macrophages and dendritic cells (Ivanov et al., 2007; Tian et al., 2007). Furthermore, HMGB1 may also bind many endogenous molecules, such as, thrombomodulin, immunoglobulin (e.g., IgG1), IL-1, or nucleosomes derived from apoptotic cells (Andersson and Tracey, 2011). In addition, these different host factors have been shown to negatively (Abeyama et al., 2005) or positively (Sha et al., 2008; Urbonaviciute et al., 2008) affect HMGB1-mediated inflammatory responses.

The activation of innate immune cells by HMGB1

Available evidence suggests that extracellular HMGB1 binds to RAGE (receptor for advanced glycation end products) and to pattern recognition receptors like TLR-2 and TLR-4 (Andersson and Tracey, 2011). Consequently, HMGB1 activates innate immune cells (91Y96) and endothelial cells (Fiuza et al., 2003; Treutiger et al., 2003) to produce proinflammatory cytokines, chemokines (Pedrazzi et al., 2007), and adhesion molecules (Fig. 4). Furthermore, one of the DNA-binding domains of HMGB1, the A box, was found to function as an antagonist of HMGB1 *in vitro* (Yang et al., 2001; Kokkola et al., 2003), whereas its B box (another DNA-binding domain) was found have the cytokine activity of full-length HMGB1 (Li et al., 2003; Messmer et al., 2004).

The inhibition of the phagocytic elimination of apoptotic neutrophils

During infection or injury, macrophages are also responsible for eliminating apoptotic cells, which they recognize using cell surface receptors for phosphatidylserine (PS). Interestingly, HMGB1 may interact with PS on surfaces of apoptotic neutrophils, and consequently inhibit the phagocytic elimination of apoptotic neutrophils by macrophages (Fig. 4) (Liu et al., 2008), which could lead excessive accumulations of late apoptotic and/or secondary necrotic cells and increase the passive leakage of HMGB1 and other DAMPs (Bell et al., 2006).

HMGB1 as a late mediator of experimental sepsis

A wide array of pro-inflammatory cytokines including TNF, IL-1, IFN-γ, and MIF (macrophage migration inhibitory factor) individually or in combination, contribute to the pathogenesis of lethal systemic inflammation (Andersson and Tracey, 2011), for example, neutralizing antibodies against TNF were found to reduce lethality in an animal model of endotoxemic/bacteremic shock (Tracey et al., 1987). However, the early kinetics of systemic TNF accumulation make it difficult to target in a clinical setting (Tracey et al., 1987), which has prompted investigations of other late proinflammatory mediators (like HMGB1) as potential therapeutic targets.

Potential therapeutic agents for sepsis

Given the limited number of effective agents available for the treatment of inflammatory diseases, it is important that other agents to found that are capable of inhibiting clinically accessible mediators. Below is a list of agents that have been shown to protect against experimental sepsis and ischemic injury, in part, by attenuating systemic or local HMGB1 accumulation (Fig. 5).

Antibodies

In animal model of sepsis, the intravenous administration of IFN-γ antibodies (1.2 mg/kg) immediately or 24 h after cecal ligation and puncture (CLP) administration decreased peritoneal and serum HMGB1 levels, and consequently attenuated CLP-induced mortality (Yin et al., 2005). In addition to cytokine-specific neutralizing antibodies, immunoglobulins (IgG) antibodies pooled from the plasma of many healthy blood donors (intravenous immunoglobulin; IVIG) have also been shown to protect against sepsis-induced lung injury and lethality by attenuating systemic HMGB1 release (Hagiwara et al., 2008a).

Anti-coagulant agents

Antithrombin inhibits the pro-coagulant activities of thrombin by interacting with heparin or related glycosaminoglycans. Although anti-thrombin III (AT-III) failed to reduce mortality in a large sepsis clinical trial (Abraham et al., 2003), it was suggested in a recent study that AT-III attenuates endotoxin-induced systemic

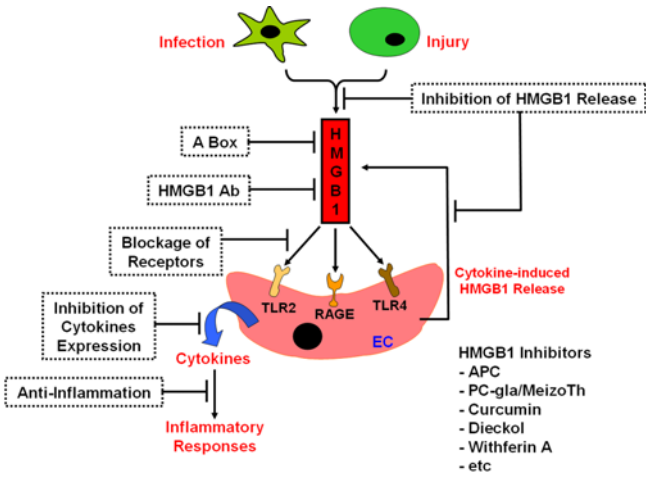

Fig. 5. Strategies targeting HMGB1 in inflammatory diseases. HMGB1 can be actively secreted by innate immune cells in response to exogenous microbial products, or passively released from injured or necrotic cells. Thus, inhibitors of HMGB1 release have therapeutic potential in sepsis and in other potentially fatal systemic inflammatory disorders. Exogenous HMGB1 can act via receptors (RAGE, TLR2, and TLR4) to stimulate the release of pro-inflammatory cytokines and elicit injurious inflammatory responses. Anti-HMGB1 treatments based on anti-HMGB1 antibodies, the specific HMGB1 antagonist A box, a HMGB1 receptor blockade, or other pharmacological agents that partially target HMGB1 have been found to be beneficial in many preclinical disease models, as described in the text.

HMGB1 accumulation and reduces endotoxemic lethality (Hagiwara et al., 2008c). Another anti-coagulant protein, thrombomodulin (TM) can bind thrombin and inhibit its pro-coagulant activities, and thus, enhances the ability to activate the plasma anticoagulant, activated protein C. Interestingly, soluble human thrombomodulin (ART-123) can bind to HMGB1 protein (Abeyama et al., 2005) and inhibit HMGB1-mediated inflammatory response. In addition, activated protein C (APC; a vitamin K-dependent plasma anticoagulant serine protease) has been approved by the Food and Drug Admin-istration for treating adult patients with severe sepsis (Bernard et al., 2001). Recently, we reported that APC inhibits the LPS-mediated release of HMGB1 and HMGB1-mediated proinflammatory signaling responses in endothelial cells by down-regulating the cell surface expressions of the HMGB1 receptors TLR2, TLR4, and RAGE (Fig. 5) (Bae and Rezaie, 2011). Interestingly, a meizothrombin derivative containing the γ-carboxyglutamic acid (Gla) domain of APC was found to potently inhibit HMGB1 signaling pathways in endothelial cells with a 20- to 50-fold higher efficacy than wild type APC (Bae and Rezaie, 2011), and to effectively cleave HMGB1 bound to TM (Bae and Rezaie, 2011).

Endogenous hormones

A number of endogenous hormones, such as, insulin (Hagiwara et al., 2008b), neuropeptides (e.g., vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP), and urocortin) (Chorny and Delgado, 2008), and ghrelin have been shown to be protective against lethal endotoxemia or sepsis partly by attenuating systemic HMGB1 accumulation. In mouse animal model of sepsis induced by CLP, the administration of urocortin was found to attenuate systemic HMGB1 accumulation, and consequently to reduce lethality (Chorny and Delgado, 2008). Ghrelin is a stomach-derived hormone that regulates appetite (levels increase before food intake and decrease afterwards). Interestingly, plasma ghrelin levels were found to be depressed in animals with septicemia (Wu et al., 2007c), and the administration of exogenous ghrelin was found to dose-dependently afford protection against sepsis-induced acute lung injury and death (Wu et al., 2007b, 2007c).

Chinese medicinal herbs

Traditional herbal medicines form the basis of folk remedies for various inflammatory ailments. After screening several dozen commonly used herbs, we found that curcumin (*Curcuma longa*), kaempferol-3-*O*-sophoroside (*Panax ginseng*), epigallocatechin gallate (*Camellia sinensis*), oleanolic acid (*Viscum album*), lycopene (*Lycopersicon esculentum*), phlorotannins (*Eisenia bicyclis*), and withaferin A (*Withania somnifera*) efficiently inhibit endotoxin-induced HMGB1 release and protected animals against lethal endotoxemia and sepsis (Fig. 6) (Kim et al., 2011, 2012a, 2012b, 2012c; Lee et al., 2012a, 2012b; Yang et al., 2012).

Curcumin (from *Curcuma longa*)

The rhizome of *Curcuma longa* has been used in indigenous medicine for the treatment of inflammatory disorders since ancient times. We found that curcumin (Fig. 6) inhibits HMGB1-mediated proinflammatory responses by increasing barrier integrity and inhibiting the expressions of cell adhesion molecules (CAMs), such as, vascular cell adhesion molecule (VCAM), intracellular adhesion molecule (ICAM), and E-selectin. Furthermore, the anti-inflammatory properties of curcumin were found to be mediated by the down-regulations of the HMGB1 receptors, TLR2 and TLR4. In fact, the inhibition of vascular endothelial inflammatory response is considered a promising target for the treatment of many vascular diseases, such as, atherosclerosis, shock, heart attack, and sepsis (Kim et al., 2011).

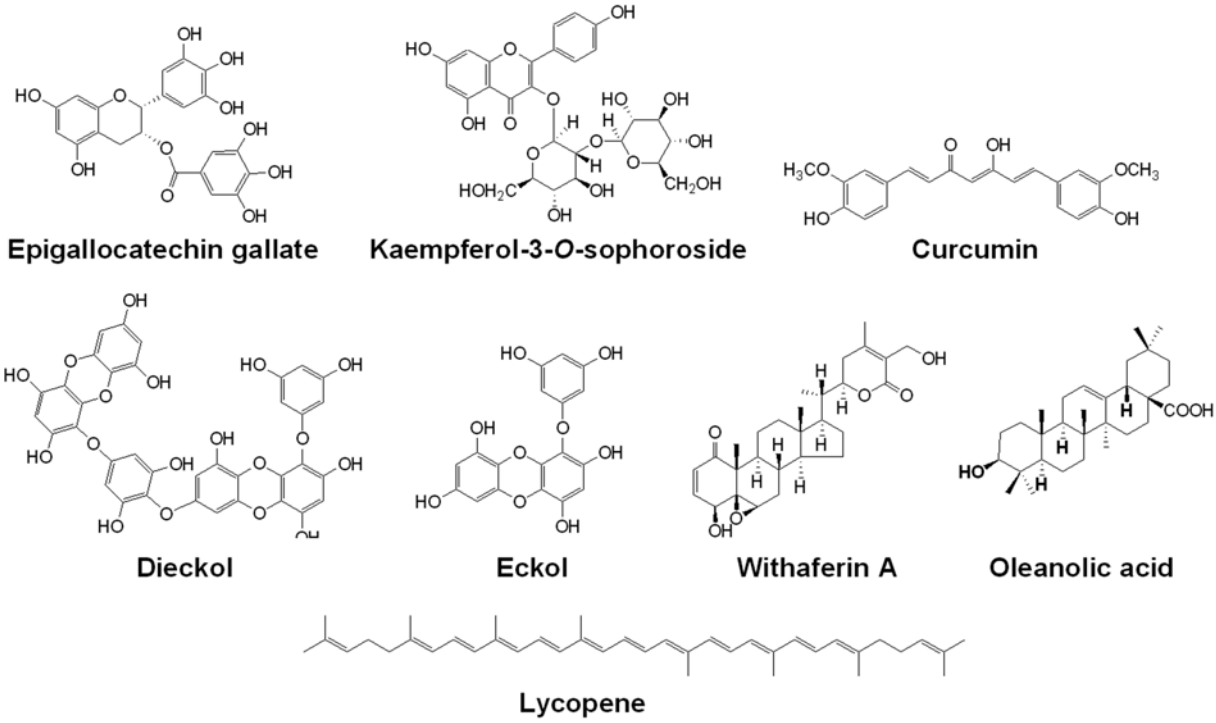

Fig. 6. Potential therapeutic herbal components for experimental sepsis.

Kaempferol-3-*O*-sophoroside (from *Panax ginseng*)

Panax ginseng of the Araliaceae is an important component of many traditional oriental herbal medicines, and has been used for thousands of years to treat disease (Zhu et al., 1999). Recently, we isolated a derivative of kaempferol, kaempferol-3-*O*-sophoroside (Fig. 6), from the leaves of cultivated mountain ginseng (P. ginseng) and showed that it inhibits the LPS-mediated release of HMGB1 and HMGB1-mediated proinflammatory responses in human endothelial cells by downregulating the cell surface expressions of the HMGB1 receptors, TLR2 and TLR4. Interestingly, kaempferol-3-O-sophoroside also protected vascular integrity and inhibited the expressions of cell adhesion molecules in HMGB1-activated human endothelial cells (Kim et al., 2012b).

Epigallocatechin gallate (EGCG, from *Camellia sinensis*)

Numerous reports have been issued on the cardiovascular benefits of green tea, and EGCG is a major constituent of the polyphenols found in green tea. Furthermore, EGCG is known to have many effects on the vasculature, including direct effects on endothelial cell function. Recently, we found that EGCG (Fig. 6) inhibited HMGB1-mediated barrier disruption by increasing barrier integrity and inhibiting ECM expression, which reduced monocyte adhesion and migration. Furthermore, EGCG inhibited HMGB1-mediated the activation of NF-κB and the production of TNF-α, the most important mediators of inflammation (Kim et al., 2012a).

Oleanolic acid (from *Viscum album*)

The mistletoe (*Viscum album*) is a semi-parasitic plant that grows on many trees worldwide. Recently, we isolated OA (Fig. 6) from *Viscum album* and found that it inhibited LPS-mediated HMGB1 release and HMGB1-mediated barrier disruption by increasing barrier integrity and inhibiting the expressions of CAMs, and thus, reducing monocyte adhesion and migration. Furthermore, OA was found to have antioxidant activity in HUVECs, to inhibit HMGB1-mediated NF-κB activation and TNF-α production, and to inhibit HMGB1-mediated RAGE expression (Yang et al., 2012).

Lycopene (from *Lycopersicon esculentum*)

Lycopene is found in tomatoes and their products, and is an linear open-chain hydrocarbon containing 11 conjugated and two non-conjugated double bonds (Britton, 1995). Recently, we found that lycopene (Fig. 6) inhibits vascular barrier permeability, the expressions of cell adhesion molecules, leukocyte adhesion, and the transendothelial migration of LPS-activated HUVECs by blocking the nuclear factor (NF)-κB activation and

TNF-α production, which suggested a potential for the treatment of vascular inflammatory diseases (Bae and Bae, 2011). In addition, lycopene was found to inhibit the LPS-mediated release of HMGB1, the HMGB1-mediated expressions of TNF-α and sPLA2-IIA, and HMGB1-mediated pro-inflammatory signaling responses in endothelial cells by down-regulating the cell surface expressions of the HMGB1 receptors, TLR-2, -4, and RAGE (Lee et al., 2012b).

Phlorotannins (from *Eisenia bicyclis*)

Eisenia bicyclis has been reported to have several biological activities, which include antioxidant, anti-tumor, anti-cancer, and bactericidal activities, and Alzheimer's disease (Nagayama et al., 2002; Okada et al., 2004). Recently, we found that a series of phlorotannins, such as eckol or dieckol (Fig. 6), inhibited HMGB1-mediated proinflammatory responses *in vitro* and *in vivo* by increasing endothelial barrier integrity, maintaining tight junctions, and inhibiting the expressions of CAMs and leukocyte adhesion and migration. In addition, we found that the hydroxyl groups in dieckol positively regulate these vascular protective effects (Kim et al., 2012c).

Withaferin A (from *Withania somnifera*)

Withania somnifera has been used to treat burns, wounds, and dermatological disorders to prevent infections (Essawi and Srour, 2000). Withaferin A is a steroidal lactone derived from *W. somnifera*, a plant that has been used for centuries to treat several inflammatory disorders (Kaileh et al., 2007). Recently, we showed that withaferin A (Fig. 6) inhibits LPS-induced HMGB1 release, LPS or HMGB1-mediated hyperpermeability, and the expressions of CAMs. We also showed withaferin A inhibits the adhesion and migration of leukocytes and that these barrier protective effects are mediated through the modulation of small GTPases and through the inhibitions of NF-κB activation and the productions of IL-6 and TNF-α (Lee *e*t al., 2012a).

CONCLUSIONS AND PERSPECTIVES

Seemingly unrelated conditions, such as, infection and injury, can converge to produce inflammatory conditions that are orchestrated by various inflammatory mediators. Extensive pre-clinical animal studies have established that HMGB1 is an early mediator of ischemic injury (Tsung et al., 2005; Liu et al., 2007; Wu et al., 2007a), and a late mediator of experimental sepsis (Wang et al., 1999a, 2004; Mantell et al., 2006). Although the therapeutic window for HMGB1-inhibiting agents is narrow, many HMGB1-inhibiting agents could rescue mice from lethal experimental sepsis even when given in a delayed fashion (e.g., 24 h after onset of sepsis). Thus, it is important to determine whether HMGB1 is a clinically feasible therapeutic target in human sepsis. For complex systemic inflammatory diseases like sepsis, it has proven to be difficult to translate success in animal studies into clinical applications, which underlies the importance of identifying the intricate mechanisms by which various agents attenuate systemic HMGB1 release.

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