Int J Clin Exp Med (2008) 1, 64-75
www.ijcem.com/IJCEM711002

Review Article

Caging a Beast in the Inflammation Arena: Use of Chinese Medicinal Herbs to Inhibit a Late Mediator of Lethal Sepsis, HMGB1

Shu Zhu[1], Wei Li[1], Jianhua Li[2], Andrew E. Sama[1], and Haichao Wang[1]

[1]Department of Emergency Medicine, North Shore University Hospital - New York University School of Medicine; and [2]The Feinstein Institute for Medical Research, Manhasset, NY 11030, USA

Received November 28, 2007; accepted January 10, 2008; available online January 20, 2008

Abstract: Sepsis refers to a systemic inflammatory response syndrome resulting from a microbial infection, which kills > 225,000 people annually in the U.S. alone. The high mortality of sepsis is partly mediated by bacterial endotoxin, which stimulates macrophages/monocytes to sequentially release early (e.g., TNF) and late (e.g., HMGB1) pro-inflammatory cytokines. Although early proinflammatory cytokines may be protective against infection, excessive accumulation of late-acting proinflammatory mediators (such as HMGB1) may sustain a potentially injurious inflammatory response. Agents capable of inhibiting HMGB1 activities (e.g., neutralizing antibodies) or release [e.g., Chinese herbs, Danggui (Angelica sinensis), Danshen (Salvia miltiorrhiza) and Green tea (Camellia sinensis)] rescue mice from lethal sepsis even when given 24 hours after onset of the disease. Here we review emerging evidence that support a critical role for extracellular HMGB1 as a late mediator of lethal sepsis, and several commonly used Chinese herbs (Danggui, Danshen and Green tea) as potential HMGB1-targeting therapeutic agents in experimental sepsis.

Key Words: Inate immune cells; cytokines; sepsis; Chinese herbs; tanshinone; catechin

Introduction

In response to microbial infection, innate immune cells (such as macrophages, monocytes, and neutrophils) constitute a front line of defense by ingesting and killing invading pathogens. If the invading pathogens are efficiently eliminated, the inflammatory response resolves normally to restore immunologic homeostasis. In contrast, inefficient pathogen clearance can lead to a rigorous inflammatory response manifested by excessive production of various proinflammatory mediators. Sepsis refers to a systemic inflammatory response syndrome resulting from a microbial infection. As a continuum of increasing clinical severity, "severe sepsis" is defined as sepsis associated with one or more acute organ dysfunctions [1]. Despite recent advances in antibiotic therapy and intensive care, sepsis is still the most common cause of death in the intensive care units, claiming approximately 225,000 victims annually in the U.S. alone.

The high mortality of sepsis is partly mediated by bacterial endotoxins (e.g., Lipopolysaccharide, LPS) [2], which activate macrophages and monocytes to release various proinflammatory mediators such as nitric oxide [3], tumor necrosis factor (TNF) [4], interleukin (IL)-1 [5], interferon (IFN)-γ 6], and macrophage migration inhibitory factor (MIF) [7]. These proinflammatory mediators, individually or in combination, contribute to the pathogenesis of lethal systemic inflammation. For instance, neutralizing antibodies to TNF, the first cytokine elaborated in inflammatory cascade, reduces lethality in an animal model of endotoxemic/bacteremic shock [4]. However, the early kinetics of TNF production makes it difficult to target in a clinical setting [4], forcing us to search for other late proinflammatory mediators that may offer a wider therapeutic window for the treatment of lethal systemic inflammatory diseases.

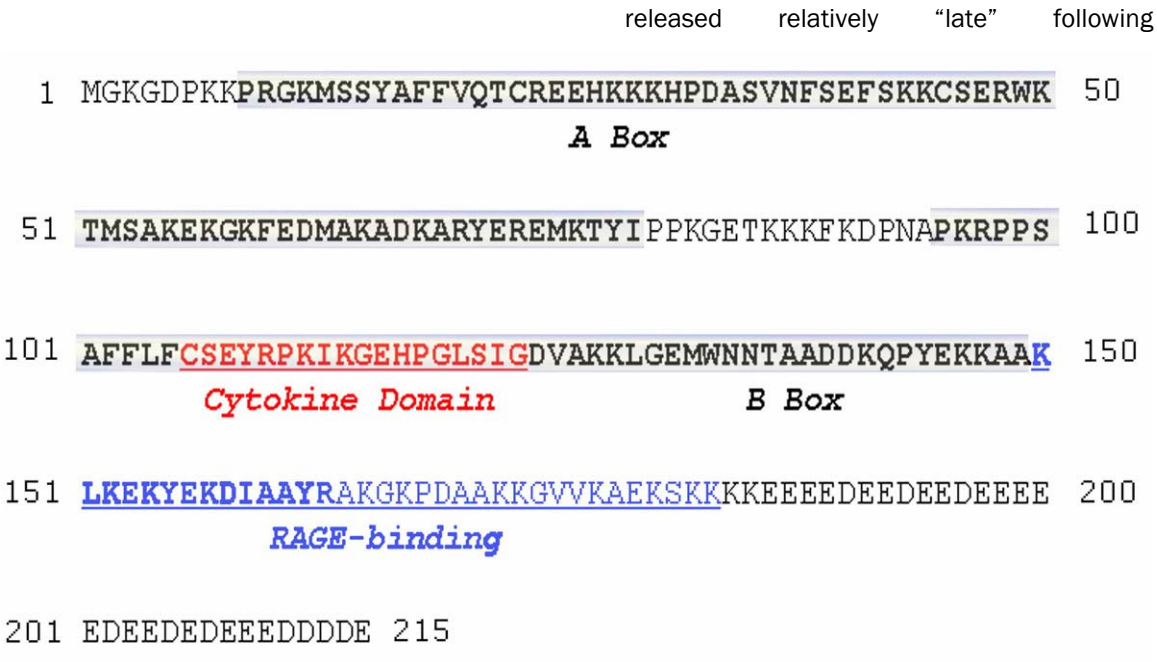

Figure 1. Amino acid sequence of human HMGB1. The N-terminal portion of HMGB1 comprises two internal repeats of a positively charged domain of about 80 amino acids (termed "HMG boxes") (shown by bold text). The cytokine-stimulating motif ("Cytokine Domain") of HMGB1 does not overlap with its RAGE-binding site, supporting the potential involvement of other cell surface receptors (such as TLR2 and TLR4) for HMGB1-mediated inflammatory responses.

Several years ago, we made the seminal observation that a ubiquitous protein, high mobility group box 1 (HMGB1), was released by activated macrophages/monocytes [8-10], and functioned as a late mediator of lethal endotoxemia [8] and sepsis [11-14]. Subsequently, we found that aqueous extracts and/or components of three Chinese herbs, Danggui (Angelica sinensis) [14], Danshen Salvia miltiorrhiza) [15] and Green tea (Camellia sinensis) [16] effectively inhibited bacterial endotoxin-induced HMGB1 release in vitro, and protected mice against lethal endotoxemia and sepsis (induced by CLP) in vivo. Here we review accumulating evidence that support a critical role for extracellular HMGB1 as a late mediator of lethal sepsis, and emerging data that suggest several Chinese medicinal herbs as potential therapeutic agents for experimental sepsis.

Discovery of HMGB1 as a Late Mediator of Lethal Sepsis

In an effort to broaden the therapeutic window for sepsis, we initiated a search for other macrophage-derived mediators that are endotoxemia. Following stimulation of macrophage cultures with bacterial endotoxin, a 30 kDa protein accumulated late in the culture medium, and was identified as the HMGB1 by N-terminal amino acid sequencing analysis [8].

Nuclear HMGB1 as a DNA-binding protein

As a non-histone nucleosomal protein, HMGB1 was purified from nuclei > 30 years ago, and termed high mobility group box 1 (HMGB1) based on its apid mobility on electrophoresis gels [17]. It is constitutively expressed in many cell types, and a large "pool" of preformed HMGB1 is stored in the nucleus due to the presence of two lysine-rich nuclear localization sequences [18, 19]. As an evolutionarily conserved protein, HMGB1 shares 100% homology (in amino acid sequence) between mouse and rat, and a 99% homology between rodent and human. HMGB1 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 (**Figure 1**). These HMG boxes enable HMGB1 to bind

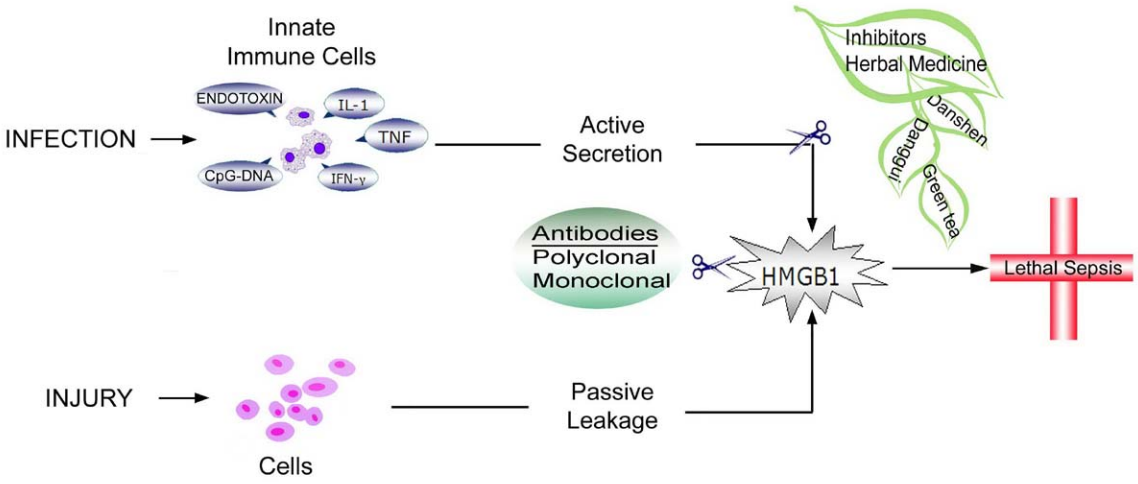

Figure 2. Schematic summary of recent development in pharmacological inhibition of HMGB1 release and action. In addition to HMGB1-specific antibodies, a number of commonly used Chinese herbs (e.g., Danggui (Angelica sinensis), Danshen (Salvia miltiorrhiza) and Green tea (Camellia sinensis)] are capable of inhibiting HMGB1 release or cytokine activities, thereby rescuing animals from lethal experimental sepsis.

chromosomal DNA and fulfill its nuclear functions including determination of nucleosomal structure and stability, and regulation of gene expression [13]. Intriguingly, HMGB1 contains consensus binding (LXCXE) motif for retinoblastoma (RB), and functions as a tumor suppressor through HMGB1/RB interaction in human breast cancer cells [20].

Extracellular release of HMGB1

In response to exogenous bacterial products (such as endotoxin or CpG-DNA) [8, 21], or endogenous inflammatory stimuli (e.g., TNF, IFN-γ, or hydrogen peroxide) [8, 9, 22], macrophages and monocytes actively release HMGB1 in a dose- and time-dependent manner (**Figure 2**). In addition, HMGB1 can be released passively from necrotic or damaged cells [23], and similarly triggers an inflammatory response.

In murine models of endotoxemia (induced by intraperitoneal administration of bacterial endotoxin, LPS) and sepsis (induced by cecal ligation and puncture, CLP), HMGB1 is first detectable in the circulation eight hours after the onset of lethal endotoxemia and sepsis, subsequently increasing to plateau levels from 16 to 32 hours [8, 11]. 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 [24].

In critically ill patients with sepsis, HMGB1 levels in the < 100 kDa serum fraction were significantly elevated, and higher in patients who did not survive than those who survived sepsis [8]. Subsequent studies indicated that levels of HMGB1 in unfractionated crude serum were persistently elevated even in patients that are recovering from severe infections [25, 26]. Unfortunately, immunoblotting analysis of HMGB1 in crude human serum could be perplexed by a few potential problems. For instance, some HMGB1-targeted antibodies could also cross-react with the light chain of human immunoglobulin on Western blots (IgG1, 25 kDa, our unpublished observations), giving potential false positive results. In contrast, many serum/plasma components (such as human IgG1) can bind to HMGB1 [27], negatively interfering with HMGB1 detection by ELISA assays. Furthermore, these serum HMGB1-binding protein(s) may also potentially modulate or mask various proinflammatory activities of HMGB1 [25, 27], adding another layer of complexity to the regulation of already intricate extracellular HMGB1 functions.

Extracellular HMGB1 as an alarmin danger signal

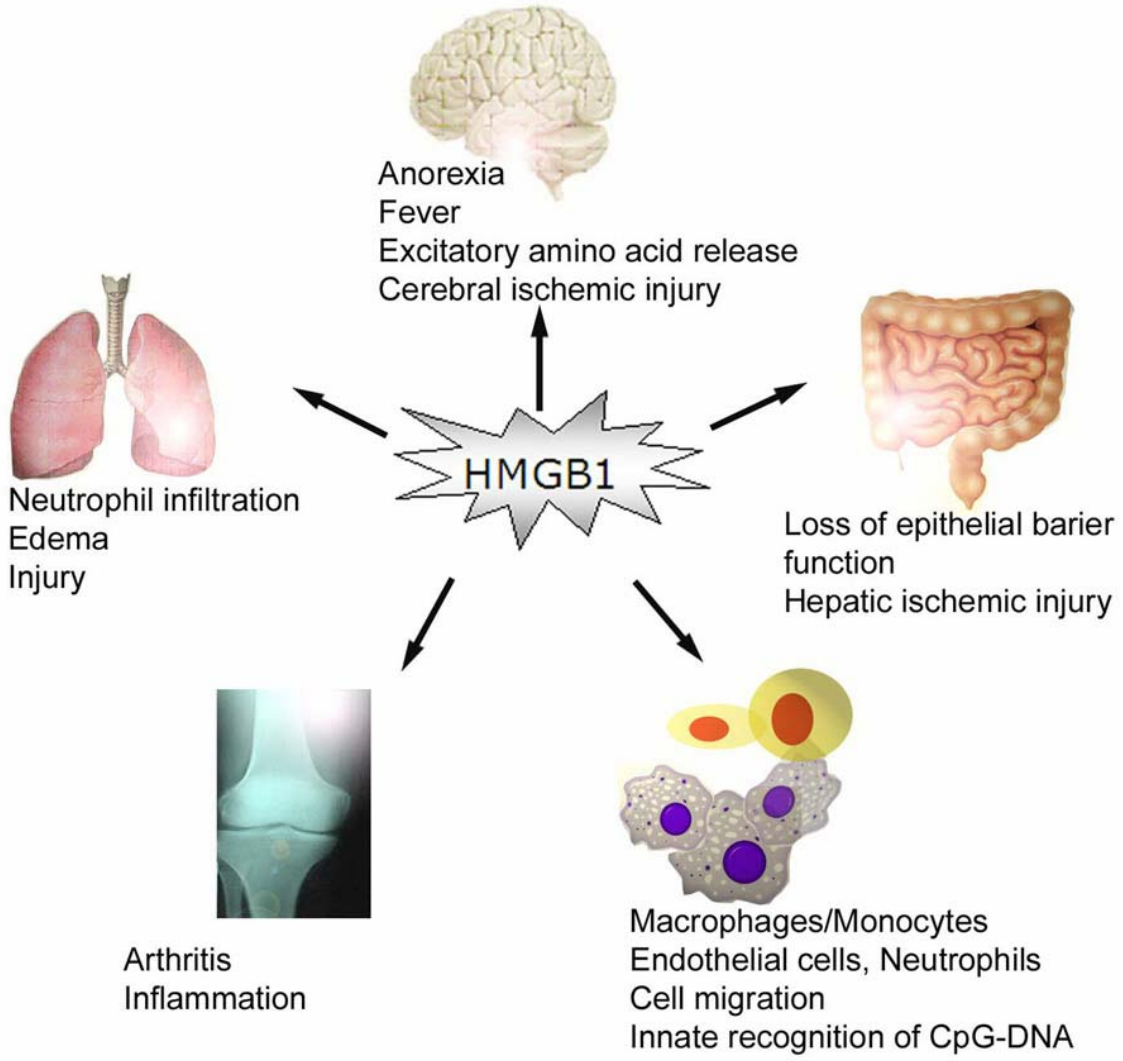

Figure 3. HMGB1 mediates proinflammatory responses. *In vivo*, administration of HMGB1 via intracerebroventricular, intratracheal, intraperitoneal, and intraarticular routes induces marked inflammatory responses. *In vitro*, HMGB1 activate various innate immune cells, and facilitate innate recognition of microbial products (such as CpG-DNA).

Recently, a number of structurally diverse, multifunctional, ubiquitous host proteins [such as high mobility group box 1 (HMGB1), and heat shock protein 72 (Hsp72)] have been categorized as "alarmins" based on several common properties [28]. First, HMGB1 is actively secreted by innate immune cells [8], and/or passively released by injured/damaged cells [23, 29]. Subsequently, extracellular HMGB1 is capable of recruiting cells to sites of infection of injury [30, 31], and facilitates innate recognition of bacterial products (e.g., CpG-DNA) by innate immune cells [21, 32]. For instance, extracellular HMGB1 can augment CpG-DNA-mediated cytokine production by innate immune cells (e.g., macrophages and dendritic cells) [21, 32], consequently facilitating innate recognition of bacterial/viral CpG-DNA to mount an effective inflammatory response.

In addition, extracellular HMGB1 binds to several cell surface receptors including the receptor for advanced glycation end products (RAGE), and the Toll-like receptor 2 (TLR2), and TLR4 [33, 34], and consequently activates

innate immune cells to produce pro-inflammatory cytokines (**Figure 3**) [33-36]. Indeed, fluorescence resonance energy transfer (FRET) analysis has demonstrated a close physical interaction between HMGB1 and TLR2 or TLR4 on macrophage cell surface within 5-15 minutes of HMGB1 incubation [35]. Intriguingly, we observed a time-dependent accumulation of exogenous HMGB1 clustering on macrophage cell surface within 4-6 hours of HMGB1 incubation [16], which correlates with the kinetics of HMGB1-induced release of proinflammatory cytokines [37]. It is plausible that engagement of exogenous HMGB1 to cell-surface receptors (such as TLR2, TLR4, and RAGE) induces clustering of ligand/receptor complexes at cell surface [16], thereby activating various innate immune cells.

Similarly, HMGB1 stimulates endothelial cells to express intracellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1), proinflammatory cytokines (e.g., TNF), and chemokines (e.g., IL-8) (**Figure 3**) [38, 39]. In the brain, exogenous HMGB1 induces release of proinflammatory cytokines [40] and excitatory amino acids (such as glutamate) [41], fever [42], and exacerbates cerebral ischemic injury (**Figure 3**) [43]. In the lung, HMGB1 induces lung neutrophil infiltration, and acute lung injury [44-46]. Focal administration of HMGB1 near the sciatic nerve induces unilateral and bilateral low threshold mechanical allodynia [47]. Similarly, intraperitoneal injection of HMGB1 increases ileal mucosal permeability, leading to bacterial translocation to mesenteric lymph nodes [48], and exacerbates hepatic ischemic injury [49].

Although highly purified eukaryotic, or bacterially produced recombinant HMGB1 has a weak proinflammatory activity by itself [21, 32, 50], it can bind to various bacterial substances (such as CpG-DNA), thereby strengthening such proinflammatory activities [21, 32]. Considered together, these studies indicate that extracellular HMGB1 can function as an alarmin signal, which alerts, recruits, and activates various innate immune cells, and consequently sustains a potentially injurious inflammatory response. Even though excessive HMGB1 may be pathogenic, low levels of HMGB1 might still be beneficial. For instance, HMGB1 is capable of attracting stem cells [31], and may be needed for tissue repair and regeneration. Therefore, like other cytokines, there may be protective advantages of extracellular HMGB1 when released at low amounts [51]. It is thus important to pharmacologically modulate, rather than abrogate, systemic HMGB1 accumulation to conquer various inflammatory diseases.

Extracellular HMGB1 as a later mediator of lethal endotoxemia and sepsis

The patho-genic role of HMGB1 as a late mediator of lethal endotoxemia was originally examined using HMGB1-specific neutralizing antibodies, which conferred significant protection against lethal endotoxemia [8], and endotoxin-induced acute lung injury (**Figure 2**) [44]. In a more clinically relevant animal model of sepsis (induced by CLP), delayed administration of HMGB1-neutralizing antibodies beginning 24 h after the onset of sepsis, dose-dependently rescued mice from lethal sepsis (**Figure 2**) [11, 52]. An increasing number of agents (anti-HMGB1 antibodies, ethyl pyruvate, stearoyl lysophosphatidyl-choline, nicotine, anti-IFN-γ antibodies) have shown efficacy in inhibiting bacterial endotoxin-induced HMGB1 release in vitro, and protecting animals against lethal endotoxemia [8] and sepsis [11, 12, 53], even when the first doses are administered 24 hours after onset of diseases [11, 12]. Notably, the first dose of the HMGB1 inhibitors were given 24 h after CLP, a time point at which mice developed clear signs of sepsis including lethargy, diarrhea, piloerection. Together, these experimental data establish HMGB1 as a late mediator of lethal endotoxemia and sepsis with a wider therapeutic window for the treatment of lethal systemic inflammatory diseases [13, 24].

Regulation of HMGB1 Release

To ensure a timely response to endotoxin, mammals have evolved an effective innate recognition system consisting of LPS-binding protein (LBP), CD14, and Toll-like receptor 4 (TLR4). When presented to CD14 by LBP, LPS is delivered to high affinity transmembrane receptors such as TLR4 [54], leading to activation of MAP kinase (e.g., p38, ERK1/2, and JNK) and NF-κB pathways, and sequential release of early (e.g., TNF) and late (e.g., HMGB1) proinflammatory cytokines. TNF is produced in vanishingly small amounts (if any at all) in quiescent macrophages/monocytes, but its transcription and translation are rapidly

up-regulated by endotoxin (LPS), leading to TNF synthesis and secretion within 1-2 hours [55]. LPS fails to induce TNF secretion in CD14-deficient macrophages [10, 56], indicating that the innate recognition system is critically important for endotoxin-induced rapid TNF release [56]. As many other cytokines, TNF contains a leader signal sequence, and is secreted via a classical endoplasmic reticulum (ER)-Golgi secretory pathway.

In contrast, HMGB1 is constitutively expressed in quiescent macrophages/monocytes, and a large "pool" of preformed HMGB1 is stored in the nucleus [9, 10]. Lacking a leader signal sequence, HMGB1 cannot be released via the classical ER-Golgi secretory pathway in response to endotoxin stimulation [8]. Instead, activated macrophages/monocytes acetylated HMGB1 at its nuclear localization sequences, leading to sequestration of HMGB1 within cytoplasmic vesicles and subsequent release into the extracellular milieu [9, 19, 57]. The LPS-stimulated HMGB1 release was only partially (by 30-50%) reduced in CD14-deficient macrophages, suggesting that innate recognition system is somewhat less critical for endotoxin-induced HMGB1 release.

In parallel, mammals have also evolved multiple negative regulatory mechanisms to counter-regulate potentially injurious inflammatory response. For instance, the central nervous system can directly and rapidly attenuate endotoxin-induced release of early (e.g., TNF) and late (e.g., HMGB1) proinflammatory cytokines through acetylcholine, the principal neurotransmitter of the vagus nerve via nicotinic cholinergic receptors [12, 58]. Another local feedback mechanism regulates inflammatory response through spermine, a ubiquitous molecule that accumulates at sites of infection or injury to function as a negative regulator of innate immune response [10, 59-62].

Exploration of Chinese Medicinal Herbs for HMGB1-Inhibiting Agents

Currently, there are two new therapies available for patients with sepsis, including the "Early Goal Directed Therapy" (EGDT) and the use of activated protein C (APC). EGDT employs extremely tight control of a number of physiological parameters (such as central venous pressure, mean arterial blood pressure, central venous oxygen saturation, blood glucose and hematocrit), which reduces septic mortality by 16% (from 46.5% to 30.5%) in a single site clinical trial [63]. On the other hand, APC marginally reduces the 28-day mortality (from 30.8% to 24.7%) [64], but is associated with a 1.5% increase in hemorrhagic complication risk. Therefore, other agents capable of inhibiting late-acting, clinically accessible mediators are still needed for the clinical management of lethal systemic inflammatory diseases.

Traditional herbal medicine has formed the basis of folk remedies for various inflammatory ailments. For instance, Danggui (Angelica sinensis) has been traditionally used to treat gynecological disorders (such as abnormal menstruation), and recently tested for efficacy in animal models of bacteria-induced pneumonia [65], carrageenan-induced edema [66], and ethanol-induced hemorrhagic tissue damage [67]. Another Chinese herb, Danshen (Saliva miltorrhiza) has been widely used in China for patients with cardiovascular disorders [68]. Similarly, green tea brewed from the leaves of the plant, Camellia sinensis, has been associated with many important health benefits, such as reduction of risk of oxidative stress and damage [69], atherosclerosis [69], cancer [70], and cardiovascular diseases [71]. After screening more than two dozen commonly used Chinese herbs, we found that aqueous extracts of Danggui, Danshen and Green tea efficiently inhibited endotoxin-induced HMGB1 release in vitro [14-16], and protected animals against lethal endotoxemia and sepsis in vivo [14].

Suppression of endotoxin-induced HMGB1 release

As the first step to elucidate immunemodulatory mechanisms of the above Chinese herbs, we examined their major anti-inflammatory components for HMGB1-inhibiting activities. Danshen contains abundant red pigments (termed tanshinone I, tanshinone IIA, and cryptotanshinone), a group of substance with medicinal value for patients with cardiovascular abnormalities [68]. Interestingly, all these tanshinones (I, IIA, and cryptotanshinone) effectively attenuated LPS-induced HMGB1 release, with estimated IC50 < 25 μM (**Figure 4**). Despite a structural resemblance (i.e., the presence of a four-fused-ring structure) between tanshinones and

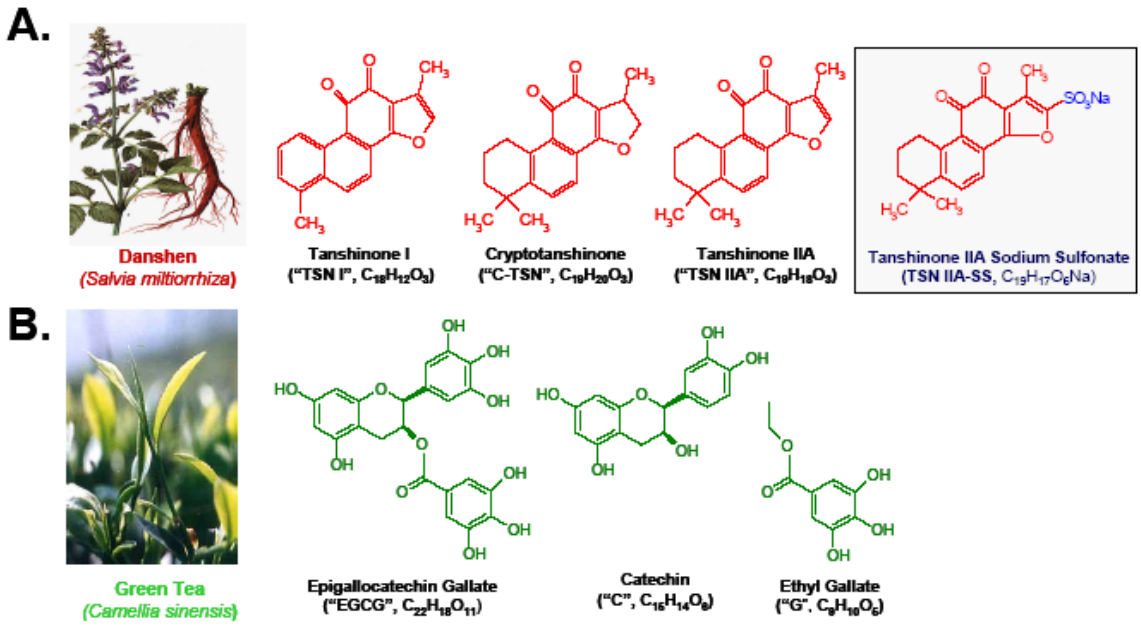

Figure 4. Structures of major components of Danshen (A) and Green tea (B). Danshen contains abundant red pigments termed tanshinone I, tanshinone IIA, and cryptotanshinone. Green tea contains catechins which harbor two or more aromatic rings (each carrying at least one aromatic hydroxyl) connected with a carbon bridge (consisting of five carbons and one oxygen).

steroidal anti-inflammatory drugs (such as dexamethasone and cortisone), these steroids failed to attenuate LPS-induced HMGB1 release [15], indicating that tanshinones and steroidal drugs exert anti-inflammatory action through distinct mechanisms.

Green tea contains a class of biologically active polyphenols called catechins, which harbor two or more aromatic rings (each carrying at least one aromatic hydroxyl) connected with a carbon bridge (consisting of five carbons and one oxygen) (**Figure 4**). Among them, EGCG accounts for 50-80% of the total catechin, representing approximately 50 mg in a single cup (100 ml) of green tea [72]. Interestingly, EGCG effectively attenuated endotoxin-induced HMGB1 release in a dose-dependent fashion, with an estimated IC50 < 1.0 μM [16]. In contrast, two relevant molecules, catechin and ethyl gallate, did not affect LPS-induced HMGB1 release, even at concentrations up to 10 μM, indicating that functional groups of both catechin and gallate are needed for EGCG's HMGB1- inhibiting properties [16].

To investigate the mechanisms by which Danggui extract and Danshen components (e.g., tanshinone IIA) inhibit HMGB1 release, we determined their effects on endotoxin-induced HMGB1 translocation – an essential step for HMGB1 release [9, 10, 57]. Danggui extract or Danshen component (TSN IIA) almost completely abrogated LPS-induced HMGB1 cytoplasmic translocation in most endotoxin-stimulated cells [14, 16], indicating that Danggui extract and Danshen component attenuate HMGB1 release by interfering with its cytoplasmic translocation.

Suppression of endotoxin-induced release of other cytokines

To better understand Danshen and Green tea's anti-inflammatory properties, we also examined their effects on LPS-induced release of other cytokines. At concentrations (15 μM) that completely abrogated LPS-induced HMGB1 release, EGCG similarly inhibited LPS-induced release of many other cytokines including IL-6, MIP-1α, MIP-1γ, MIP-2, RANTES, KC, MCP1, and CXCL16 [16]. In sharp contrast, a watersoluble derivative (sodium sulfonate) of tanshinone IIA, TSN IIA-SS (**Figure 4**), at concentrations (100 μM) that completely

abrogated LPS-induced HMGB1 release, did not suppress LPS-induced release of most cytokines, and only partially attenuated LPS-induced release of IL-12p70, IL-1α, platelet factor 4 (PF-4), and MCP-5 [15]. Taken together, these data indicate that Danshen and Green tea components inhibit several common mediators (e.g., HMGB1), and at the same time exhibit distinct specificities with respect to other cytokines.

Protection against lethal endotoxemia and sepsis

In light of the capacity of aqueous extracts and components of Danggui, Danshen and Green tea in attenuating LPS-induced HMGB1 release, we explored their efficacy in an animal model of lethal endotoxemia. Repeated administration of Danggui extract, TSN IIA-SS and EGCG conferred a dose-dependent protection against lethal endo-toxemia [14-16]. More importantly, in animal models of experimental sepsis induced by cecal ligation and puncture, repeated adminis-tration of the above agents beginning at +24 h, followed by additional doses at +48, +72 and + 96 h after the onset of sepsis, dose-dependently rescued mice from lethal sepsis (**Figure 2**) [14-16].

To gain insight into the mechanisms underlying herbal extract or component-mediated protection against lethal sepsis, we evaluated their effects on systemic accumulation of various cytokines. Delayed administration of Danggui extract, TNS IIA-SS, or EGCG did not attenuate circulating levels of TNF or nitric oxide at 52 h after the onset of sepsis (data not shown), but dose-dependently attenuated circulating HMGB1 levels in septic mice [14-16]. Furthermore, delayed adminis-tration of EGCG markedly attenuated circu-lating levels of IL-6 and KC [16] - two most reliable surrogate markers of experimental sepsis that can predict outcome [73]. Considered together, these experimental data indicate that these herbal extracts and/or components protect mice against lethal sepsis partly by attenuating systemic accumulation of a late proinflammatory mediator, HMGB1. At present, our experimental data can not exclude the possibility that herbal extracts and/or components confer protection against lethal sepsis through additional unknown mechanisms. Therefore, future studies are needed to better understand the protective mechanisms underlying Chinese herbal medicinal herb-mediated protective effects.

In light of the clinical use of TSN IIA-SS in China for patients with cardiovascular disorders [68], we also determined whether it improves cardiovascular function in septic animals. Administration of TSN IIA-SS did not significantly affect the mean arterial blood pressure, but slightly reduced the heart rate (from 378.3 ± 25.1 to 334.1 ± 25.8 beats/minutes, $P < 0.05$). More importantly, it dosedependently reduced total peripheral vascular resistance, and yet significantly increased cardiac stroke volume, and cardiac output [15]. As an important organ frequently compromised by sepsis and septic shock, poor cardiac output as a consequence of depressed myocardial function may contribute to the pathogenesis of lethal sepsis or septic shock [74]. The dual effects of TSN IIA-SS in attenuating late inflammatory response and improving cardiovascular function make it a promising therapeutic agent for patients with sepsis.

Suppression of HMGB1 cytokine activities

To elucidate additional mechanisms underlying EGCG-mediated protection, we determined whether Green tea component (EGCG) inhibits HMGB1-mediated inflamematory response. Indeed, EGCG dose-dependently inhibited HMGB1- induced release of TNF, IL-6, and nitric oxide in macrophage cultures [16]. Furthermore, EGCG effectively inhibited HMGB1-induced release of IL-6 release, even when it was given 2-4 hours after HMGB1 stimulation. Despite the fact that EGCG failed to inhibit LPS-induced nitric oxide, it dose-dependently suppressed HMGB1-induced release of nitric oxide in macrophage cultures, supporting the notion that LPS and HMGB1 use distinct mechanisms to activate innate immune cells [37, 75]. Taken together, these data suggest that EGCG confers protection against lethal sepsis partly by inhibiting HMGB1 cytokine activities.

To elucidate the mechanism by which EGCG attenuates HMGB1-mediated cytokine production, we determined its effect on macrophage cell surface accumulation/ clustering of exogenous HMGB1. Cell surface accumulation/clustering of exogenous (biotin-labeled) HMGB1, as indicated by streptavidin-conjugated Alexa 594 fluorescence, was noted at 4-6 h post HMGB1 treatment [16]. In the

presence of EGCG (10 μM), the cell surface accumulation/clustering of exogenous HMGB1 was almost completely eliminated [16], suggesting that EGCG inhibits HMGB1 cytokine activities by preventing its cell surface accumulation/clustering.

Conclusion

A ubiquitous nuclear protein, HMGB1, is released by activated macrophages/ monocytes, and functions as a late mediator of lethal endotoxemia and sepsis. First, circulating HMGB1 levels are elevated in a delayed fashion in endotoxemic and septic animals. Second, administration of exogenous HMGB1 to mice induces fever, derangement of intestinal barrier function, and tissue injury. Third, administration of anti-HMGB1 antibodies or inhibitors (e.g., Chinese medicinal herbs, Danggui, Danshen, and Green tea) protects mice against lethal endotoxemia, and rescues mice from lethal experimental sepsis even when the first doses are given 24 hours after onset of sepsis. Taken together, these experimental data establish HMGB1 as a late mediator of lethal endotoxemia and sepsis with a wider therapeutic window, and support several commonly used Chinese herbs (e.g., Danggui, Danshen and Green tea) as potential therapeutic agents for experimental sepsis. The downstream or "late" action of HMGB1 is a marked departure from the early activities of TNF and other classical proinflammatory cytokines, and has significant implications for understanding and manipulating innate immune responses. Thus, future studies are needed to further explore the therapeutic potential of Chinese herbal medicine in the clinical management of human sepsis.

Acknowledgments

The studies summarized in this review were supported in part by NIH grants R01-GM063075 and R01GM070817 (to HW).

Address correspondence to: Haichao Wang, PhD, Department of Emergency Medicine, North Shore University Hospital - New York University School of Medicine, 350 Community Drive, Manhasset, NY 11030, Fax: (516) 562-1022, E-mail: hwang@nshs.edu.

References

[1] Riedemann NC, Guo RF and Ward PA. The enigma of sepsis. J Clin Invest 2003;112:460-467.

[2] Ayala A, Song GY, Chung CS, Redmond KM and Chaudry IH. Immune depression in polymicrobial sepsis: the role of necrotic (injured) tissue and endotoxin. Crit Care Med 2000;28:2949-2955.

[3] Dinapoli MR, Calderon CL and Lopez DM. The altered tumoricidal capacity of macrophages isolated from tumor-bearing mice is related to reduce expression of the inducible nitric oxide synthase gene. J Exp Med 1996;183:1323-1329.

[4] Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF and Cerami A. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 1987;330:662-664.

[5] Dinarello CA and Thompson RC. Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. Immunol Today 1991;12:404-410.

[6] Heinzel FP. The role of IFN-gamma in the pathology of experimental endotoxemia. J Immunol 1990;145:2920-2924.

[7] Calandra T, Echtenacher B, Roy DL, Pugin J, Metz CN, Hultner L, Heumann D, Mannel D, Bucala R and Glauser MP. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat Med 2000;6:164-170.

[8] Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama AE and Tracey KJ. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999;285:248-251.

[9] Rendon-Mitchell B, Ochani M, Li J, Han J, Wang H, Yang H, Susarla S, Czura C, Mitchell RA, Chen G, Sama AE, Tracey KJ and Wang H. IFN-gamma Induces High Mobility Group Box 1 Protein Release Partly Through a TNF-Dependent Mechanism. J Immunol 2003;170:3890-3897.

[10] Chen G, Li J, Ochani M, Rendon-Mitchell B, Qiang X, Susarla S, Ulloa L, Yang H, Fan S, Goyert SM, Wang P, Tracey KJ, Sama AE and Wang H. Bacterial endotoxin stimulates macrophages to release HMGB1 partly through CD14- and TNF-dependent mechanisms. J Leukoc Biol 2004;76:994-1001.

[11] Yang H, Ochani M, Li J, Qiang X, Tanovic M, Harris HE, Susarla SM, Ulloa L, Wang H, DiRaimo R, Czura CJ, Wang H, Roth J, Warren HS, Fink MP, Fenton MJ, Andersson U and Tracey KJ. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. Proc Natl Acad Sci U S A 2004;101:296-301.

[12] Wang H, Liao H, Ochani M, Justiniani M, Lin X,

Yang L, Al Abed Y, Wang H, Metz C, Miller EJ, Tracey KJ and Ulloa L. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat Med 2004;10:1216-1221.

[13] Wang H, Yang H and Tracey KJ. Extracellula role of HMGB1 in inflammation and sepsis. J Intern Med 2004;255:320-331.

[14] Wang H, Li W, Li J, Rendon-Mitchell B, Ochani M, Ashok M, Yang L, Yang H, Tracey KJ, Wang P and Sama AE. The Aqueous Extract of a Popular Herbal Nutrient Supplement, Angelica sinensis, Protects Mice against Lethal Endotoxemia and Sepsis. J Nutr 2006;136:360-365.

[15] Li W, Li J, Ashok M, Wu R, Chen D, Yang L, Yang H, Tracey KJ, Wang P, Sama AE and Wang H. A cardiovascular drug rescues mice from lethal sepsis by selectively attenuating a late-acting proinflammatory mediator, high mobility group box 1. J Immunol 2007;178:3856- 3864.

[16] Li W, Ashok M, Li J, Yang H, Sama AE and Wang H. A Major Ingredient of Green Tea Rescues Mice from Lethal Sepsis Partly by Inhibiting HMGB1. PLoS ONE 2007;2:e1153.

[17] Johns EW. History, Definitions and Problems. In: Johns EW, ed. The HMG Chromosomal Proteins. London: Academic Press Inc. (London) Ltd, 1982:1-8.

[18] Chen G, Li J, Qiang X, Czura CJ, Ochani M, Ochani K, Ulloa L, Yang H, Tracey,KJ, Wang P, Sama AE and Wang H. Suppression of HMGB1 release by stearoyl lysophosphatidylcholine:an additional mechanism for its therapeutic effects in experimental sepsis. J Lipid Res 2005;46:623-627.

[19] Bonaldi T, Talamo F, Scaffidi P, Ferrera D, Porto A, Bachi A, Rubartelli A, Agresti A and Bianchi ME. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. EMBO J 2003;22:5551-5560.

[20] Jiao Y, Wang H and Fan SJ. Growth suppression and radiosensitivity increase by HMGB1 in breast cancer. Acta Pharmacol Sin 2007;28:1957-1967.

[21] Ivanov S, Dragoi AM, Wang X, Dallacosta C, Louten J, Musco G, Sitia G, Yap GS, Wan Y, Biron CA, Bianchi ME, Wang H and Chu WM. A novel role for HMGB1 in TLR9-mediated inflammatory responses to CpG-DNA. Blood 2007;10(6): 1970-1981.

[22] Tang D, Shi Y, Kang R, Li T, Xiao W, Wang H and Xiao X. Hydrogen peroxide stimulates macrophages and monocytes to actively release HMGB1. J Leukoc Biol 2007;81:741-747.

[23] Scaffidi P, Misteli T and Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002;418:191-195.

[24] Wang H, Yang H, Czura CJ, Sama AE and Tracey KJ. HMGB1 as a Late Mediator of Lethal Systemic Inflammation. Am J Respir Crit Care Med 2001;164:1768-1773.

[25] Sunden-Cullberg J, Norrby-Teglund A, Rouhiainen A, Rauvala H, Herman G, Tracey KJ, Lee ML, Andersson J, Tokics L, Treutiger CJ. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. Crit Care Med 2005;33:564- 573.

[26] Angus DC, Yang L, Kong L, Kellum JA, Delude RL, Tracey KJ, Weissfeld L. Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis. Crit Care Med 2007;35:1061-1067.

[27] Urbonaviciute V, Furnrohr BG, Weber C, Haslbeck M, Wilhelm S, Herrmann M, Voll RE. Factors masking HMGB1 in human serum an plasma. J Leukoc Biol 2007;81:67-74.

[28] Oppenheim JJ, Yang D. Alarmins: chemotactic activators of immune responses. Curr Opin Immunol 2005;17:359-365.

[29] Tsung A, Klune JR, Zhang X, Jeyabalan G, Cao Z, Peng X, Stolz DB, Geller DA, Rosengart MR, Billiar TR. HMGB1 release induced by liver ischemia involves Toll-like receptor 4 dependent reactive oxygen species production and calcium-mediated signaling. J Exp Med 2007;204:2913-2923.

[30] Degryse B, Bonaldi T, Scaffidi P, Muller S, Resnati M, Sanvito F, Arrigoni G, Bianchi ME. The high mobility group (HMG) boxes of the nuclear protein HMG1 induce chemotaxis and cytoskeleton reorganization in rat smooth muscle cells. J Cell Biol 2001;152:1197-1206.

[31] Palumbo R, Sampaolesi M, De Marchis F, Tonlorenzi R, Colombetti S, Mondino A, Cossu G, Bianchi ME. Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. J Cell Biol 2004;164:441-449.

[32] Tian J, Avalos AM, Mao SY, Chen B, Senthil K, Wu H, Parroche P, Drabic S, Golenbock D, Sirois C, Hua J, An LL, Audoly L, La Rosa,G, Bierhaus A, Naworth P, Marshak-Rothstein A, Crow MK, Fitzgerald KA, Latz E, Kiener PA, Coyle AJ. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. Nat Immunol 2007;8:487-496.

[33] Park JS, Svetkauskaite D, He , Kim JY, Strassheim D, Ishizaka A, Abraham E. Involvement of TLR 2 and TLR 4 in cellular activation by high mobility group box 1 protein (HMGB1). J Biol Chem 2004;279:7370-7377.

[34] Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ, Fenton MJ, Tracey KJ and Yang H. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock 2006;26:174-179.

[35] Park JS, Gamboni-Robertson F, He Q Svetkauskaite D, Kim JY, Strassheim D, Sohn JW, Yamada S, Maruyama I, Banerjee A, Ishizaka A and Abraham E. High mobility group box 1 protein interacts with multiple Toll-like

receptors. Am J Physiol Cell Physiol 2006;290:C917- C924.
[36] Kokkola R, Andersson A, Mullins G, Ostberg T, Treutiger CJ, Arnold B, Nawroth P, Andersson U, Harris RA and Harris HE. RAGE is the Major Receptor for the Proinflammatory Activity of HMGB1 in Rodent Macrophages. Scand J Immunol 2005;61:1-9.
[37] Andersson U, Wang H, Palmblad K, Aveberger AC, Bloom O, Erlandsson-Harris H, Janson A, Kokkola R, Zhang M, Yang H and Tracey KJ. High Mobility Group 1 Protein (HMG-1) Stimulates Proinflammatory Cytokine Synthesis in Human Monocytes. J Exp Med 2000;192:565-570.
[38] Fiuza C, Bustin M, Talwar S, Tropea M, Gerstenberger E, Shelhamer JH and Suffredini AF. Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. Blood 2003;101:2652-2660.
[39] Treutiger CJ, Mullins GE, Johansson AS, Rouhiainen A, Rauvala HM, Erlandsson-Harris H, Andersson U, Yang H, Tracey KJ, Andersson J and Palmblad JE. High mobility group 1 Bbox mediates activation of human endothelium. J Intern Med 2003;254:375-385.
[40] Agnello D, Wang H, Yang H, Tracey KJ and Ghezzi P. HMGB1, a DNA-binding protein with cytokine activity, induces brain TNF and IL-6 production, and mediates anorexia and taste aversion. Cytokine 2002;18:231-236.
[41] Pedrazzi M, Raiteri L, Bonanno G, Patrone M, Ledda S, Passalacqua M, Milanese M, Melloni E, Raiteri M, Pontremoli S and Sparatore B. Stimulation of excitatory amino acid release from adult mouse brain glia subcellular particles by high mobility group box 1 protein. J Neurochem 2006;99:827-838.
[42] O'Connor KA, Hansen MK, Rachal PC, Deak MM, Biedenkapp JC, Milligan ED, Johnson JD, Wang H, Maier SF, Tracey KJ and Watkins LR. Further characterization of high mobility group box 1 (HMGB1) as a proinflammatory cytokine: central nervous system effects. Cytokine 2003;24:254-265.
[43] Liu K, Mori S, Takahashi HK, Tomono Y, Wak H, Kanke T, Sato Y, Hiraga N, Adachi N, Yoshino T and Nishibori M. Anti-high mobility group box 1 monoclonal antibody ameliorates brain infarction induced by transient ischemia in rats. FASEB J 2007 (in press).
[44] Abraham E, Arcaroli J, Carmody A, Wang H and Tracey KJ. HMG-1 as a mediator of acute lun inflammation. J Immunol 2000;165:2950-2954.
[45] Ueno H, Matsuda T, Hashimoto S, Amaya F Kitamura Y, Tanaka M, Kobayashi A, Maruyama I, Yamada S, Hasegawa N, Soejima J, Koh H and Ishizaka A. Contributions of high mobility group box protein in experimental and clinical acute lung injury. Am J Respir Crit Care Med 2004;170:1310-1316.
[46] Lin X, Yang H, Sakuragi T, Hu M, Mantell LL Hayashi S, Al Abed Y, Tracey KJ, Ulloa L and Miller EJ. {alpha}-Chemokine receptor blockade reduces high mobility group box 1 protein-induced lung inflammation and injury and improves survival in sepsis. Am J Physiol Lung Cell Mol Physiol 2005;289:L583-L590.
[47] Chacur M, Milligan ED, Gazda LS, Armstrong C, Wang H, Tracey KJ, Maier SF and Watkins LR. A new model of sciatic inflammatory neuritis (SIN): induction of unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats. Pain 2001;94:231-244.
[48] Sappington PL, Yang R, Yang H, Tracey KJ, Delude RL and Fink MP. HMGB1 B box increases the permeability of Caco-2 enterocytic monolayers and impairs intestinal barrier function in mice. Gastroenterology 2002;123:790-802.
[49] Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA and Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med 2005;201:1135-1143.
[50] Rouhiainen A, Tumova S, Valmu L, Kalkkinen N, Rauvala H. Pivotal advance: analysis of proinflammatory activity of highly purified eukaryotic recombinant HMGB1 (amphoterin). J Leukoc Biol 2007;81:49-58.
[51] Li W, Sama AE and Wang H. Role of HMGB1 in cardiovascular diseases. Curr Opin Pharmacol 2006;6:130-135.
[52] Qin S, Wang H, Yuan R, Li H, Ochani M, Ochani K, Rosas-Ballina M, Czura CJ, Huston JM, Miller E, Lin X, Sherry B, Kumar A, Larosa G, Newman W, Tracey KJ and Yang H. Role of HMGB1 in apoptosis-mediated sepsis lethality. J Exp Med 2006;203:1637-1642.
[53] Pahuja M, Tran C, Wang H and Yin K. Alveolar macrophage suppression in sepsis is associated with high mobility group box 1 transmigration. Shock 2007 (in press).
[54] Poltorak A, He X, Smirnova I, Liu MY, Huffel CV, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 1998;282:2085-2088.
[55] Wang H, Czura CJ and Tracey K J. TNF. In: Thomson A, Lotze MT, eds. The Cytokine Handbook. Oxford, Academic Press, 2003:837-860.
[56] Haziot A, Ferrero E, Kontgen F, Hijiya N, Yamamoto S, Silver J, Stewart CL and Goyert SM. Resistance to endotoxin shock and reduced dissemination of gram-negative bacteria in CD14- deficient mice. Immunity 1996;4:407-414.
[57] Gardella S, Andrei C, Ferrera D, Lotti LV, Torrisi MR, Bianchi ME and Rubartelli A. The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. EMBO Rep 2002;3:955-1001.

[58] Borovikova LV, Ivanova S, Zhang M, Yang H Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW and Tracey KJ. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 2000;405:458-462.
[59] Zhang M, Caragine T, Wang H, Cohen PS, Botchkina G, Soda K, Bianchi M, Ulrich P, Cerami A, Sherry B and Tracey KJ. Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells: a counterregulatory mechanism that restrains the immune response. J Exp Med 1997;185:1759-1768.
[60] Zhang M, Borovikova LV, Wang H, Metz C and Tracey KJ. Spermine inhibition of monocyte activation and inflammation. Mol Med 1999;5:595-605.
[61] Wang H, Zhang M, Bianchi M, Sherry B, Sama AE and Tracey KJ. Fetuin (alpha2-HSglycoprotein) opsonizes cationic macrophagedeactivating molecules. Proc Natl Acad Sci U S A 1998;95:14429-14434.
[62] Zhang M, Wang H and Tracey KJ. Regulation of macrophage activation and inflammation by spermine: a new chapter in an old story. Crit Care Med 2000;28:N60-N66.
[63] Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E and Tomlanovich M. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med 2001;345:1368-1377.
[64] Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW and Fisher CJJ. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001;344:699-709.
[65] Song ZJ, Johansen HK, Moser C, Faber V Kharazmi A, Rygaard J and Hoiby N. Effects of Radix Angelicae sinensis and shuanghuanglian on a rat model of chronic Pseudomonas aeruginosa pneumonia. Chin Med Sci J 2000;15:83-88.
[66] Hu H, Hang B and Wang P. Anti-inflammatory effect of radix Angelicae sinensis. Zhongguo Zhong Yao Za Zhi 1991;16:684-6, 704.
[67] Cho CH, Mei QB, Shang P, Lee SS, So HL, Guo X and Li Y. Study of the gastrointestinal protective effects of polysaccharides from Angelica sinensis in rats. Planta Med 2000;66 (4): 348-351.
[68] Ji XY, Tan BK and Zhu YZ. Salvia miltiorrhiza and ischemic diseases. Acta Pharmacol Sin 2000;21:1089-1094.
[69] Frei B and Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr 2003;133:3275S-3284S.
[70] Crespy V and Williamson G. A review of the health effects of green tea catechins in in vivo animal models. J Nutr 2004;134:3431S-3440S.
[71] Vita JA. Tea consumption and cardiovascular disease: effects on endothelial function. J Nutr 2003;133:3293S-3297S.
[72] Graham HN. Green tea composition, consumption, and polyphenol chemistry. Prev Med 1992;21:334-350.
[73] Osuchowski MF, Welch K, Siddiqui J and Remick DG. Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continuum in sepsis and predict mortality. J Immunol 2006;177:1967-1974.
[74] Makwana N and Baines PB. Myocardial dysfunction in meningococcal septic shock. Curr Opin Crit Care 2005;11:418-423.
[75] Park JS, Arcaroli J, Yum HK, Yang H, Wang H, Yang KY, Choe KH, Strassheim D, Pitts TM, Tracey KJ and Abraham E. Activation of gene expression in human neutrophils by high mobility group box 1 protein. Am J Physiol Cell Physiol 2003;284:C870-C879.