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NOVEL HMGB1-INHIBITING THERAPEUTIC AGENTS FOR EXPERIMENTAL SEPSIS

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Abstract

Sepsis refers to a systemic inflammatory response syndrome resulting from a microbial infection. The inflammatory response is partly mediated by innate immune cells (such as macrophages, monocytes and neutrophils), which not only ingest and eliminate invading pathogens, but also initiate an inflammatory response by producing early (e.g., TNF and IFN-gamma) and late (e.g., HMGB1) proinflammatory cytokines. Here we briefly review emerging evidence that support extracellular HMGB1 as a late mediator of experimental sepsis, and discuss therapeutic potential of several HMGB1-inhibiting agents (including neutralizing antibodies and steroid-like tanshinones) in experimental sepsis.

Keywords

innate immune cells; phagocytes; inflammation; cytokines; sepsis; antibodies; HMGB1; tanshinones

INTRODUCTION

In response to microbial infection or injury, the host's innate immune system mounts an immediate biological response - termed "inflammation" ("set on fire", in Greek) - to remove invading pathogens and to heal the wound (1). Upon effective pathogen elimination and tissue repair, inflammation normally resolves to restore immunologic homeostasis (2). Otherwise, exogenous pathogens or endogenous proinflammatory mediators can leak into the blood stream, triggering a systemic inflammatory response that may lead to sepsis (1) (Fig. 1). Sepsis refers to a systemic inflammatory response syndrome resulting from a microbial infection. As a continuum of increasing clinical severity, sepsis can progress into "severe sepsis" or "septic shock" (3). Here we briefly review the prevailing theories of sepsis as an uncontrolled systemic inflammatory response, and discuss potential therapeutic agents that target a late mediator of experimental sepsis.

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INNATE IMMUNE RESPONSE AGAINST MICROBIAL INFECTION

The innate immune cells (such as macrophages, monocytes and neutrophils) are responsible not only for eliminating invading pathogens, but also for initiating an inflammatory response (1).

Elimination of invading pathogens

Monocytes and neutrophils continuously patrol the body to search for invading pathogens or damaged tissues, and infiltrate into infected/injured tissues upon detecting exogenous microbial products or endogenous chemotactic factors (4). Neutrophils are usually the first to arrive at the infection site, and kill more invading bacteria than other phagocytes (Fig. 1) (5). After engulfing and killing bacteria, however, neutrophils exhaust intracellular enzymes, and subsequently undergo apoptotic cell death. In contrast, monocytes can differentiate into tissue-specific resident macrophages (such as Kupffer cells) once reaching extravascular tissues. Macrophages recognize pathogens or apoptotic cells through opsonins (such as complement or antibodies) (6) or cell surface receptors for phosphatidylserine (PS) (7). After engulfing pathogens or damaged cells, phagocytes eliminate them through reactive oxygen species and hydrolytic enzymes (Fig. 1) (8).

Initiation of inflammatory responses

Innate immune cells are equipped with pattern recognition receptors (such as Toll-like receptor, TLR 2, 4, and 9) (9–11), which specifically recognize molecules shared by a group of related microbes called pathogen-associated molecular patterns (PAMPs). Upon recognition of various PAMPs (such as bacterial peptidoglycan, endotoxin, and CpG-DNA) (11,12), innate immune cells release a wide array of cytokines and chemokines (13–15). Although an appropriate local inflammation is required to defend against infection or injury, an uncontrolled systemic inflammation may contribute to the pathogenesis of lethal inflammation diseases (such as sepsis).

SEPSIS AS A DYSREGULATED SYSTEMIC INFLAMMATORY RESPONSE

The prevailing theories of sepsis as an dysregulated systemic inflammatory response are supported by extensive studies employing various animal models of sepsis, including endotoxemia and peritonitis induced by cecal ligation and puncture (CLP) (1,16).

Early pro-inflammatory mediators of experimental sepsis

In animal models of sepsis, a wide array of pro-inflammatory mediators including TNF (17), interleukin (IL)-1 (18), interferon (IFN)- γ (19), and macrophage migration inhibitory factor (MIF) (20,21) individually or in combination, contribute to the pathogenesis of lethal systemic inflammation. For instance, neutralizing antibodies against endotoxin (22) or TNF (17), reduces lethality in an animal model of endotoxemic/bacteremic shock. However, the early kinetics of systemic TNF accumulation makes it difficult to target in clinical setting (17), prompting a search for other late proinflammatory mediators that may offer a wider therapeutic window (Fig. 2).

Discovery of HMGB1 as a late mediator of experimental sepsis

In an attempt to broaden the therapeutic window for sepsis, we initiated a search for other macrophage-derived putative mediator released relatively “late” after onset of endotoxemia (23). We stimulated macrophage cultures with bacterial endotoxin, and screened the conditioned media for proteins appearing after 16 hours. Endotoxin induced the appearance of a 30-kDa protein in the conditioned media that was not detectable at earlier time points. The N-terminal amino acid sequence of this 30-kDa protein (i.e., G-K-G-D-P-K-K-P-R-G-

K-M-S-S) was identical to a non-histone nucleosomal protein termed “high mobility group 1” (HMG-1) (23–25).

Nuclear HMGB1 as a transcription factor—HMG-1 was first purified from nuclei approximately 40 years ago, and termed “high mobility group” (HMG) protein because of its rapid mobility on electrophoresis gels (26). Recently, HMG-1 was renamed as high mobility group box 1 (HMGB1) by a nomenclature committee (27). It is constitutively expressed in many types of cells, and a large “pool” of preformed HMGB1 is stored in the nucleus, owing to the presence of two lysine-rich nuclear localization sequences (28). 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 (24,25,29). It contains two internal repeats of positively charged domains (“HMG boxes” known as “A box” and “B box”) in the N-terminus, and a continuous stretch of negatively charged (aspartic and glutamic acid) residues in the C-terminus. These HMG boxes enable HMGB1 to bind chromosomal DNA, and fulfill its nuclear functions including determination of nucleosomal structure and stability, and regulation of gene expression (30).

Delayed systemic HMGB1 accumulation—In murine models of endotoxemia and sepsis, HMGB1 is first detectable in the circulation eight hours after the onset of diseases, subsequently increasing to plateau levels from 16 to 32 hours (23,31). Meanwhile, tissue HMGB1 mRNA levels are increased in various tissues such as muscle, liver, and lung following endotoxemia (32) or burn-induced sepsis (33). This late appearance of circulating HMGB1 precedes and parallels with the onset of animal lethality from endotoxemia or sepsis, and distinguishes itself from TNF and other early proinflammatory cytokines (Fig. 2) (34). In septic patients, circulating HMGB1 levels were also elevated (23,35,36), although its levels in un-fractionated crude serum samples did not correlate with disease severity (35,36). However, HMGB1 levels in the low molecular weight (M.W. < 100 kDa) sub-fraction (following ultrafiltration of serum samples through filters with defined M.W. cut-off) correlated well with the lethal outcome of human sepsis (1,23). This observation suggested a possibility that HMGB1 may interact with other serum components such as thrombomodulin (37), immunoglobulin (e.g., IgG1) (38) to form large (> 100 kDa) complexes (1).

Protective effects of anti-HMGB1 antibodies—The pathogenic role of HMGB1 as a late mediator of lethal endotoxemia was originally examined using HMGB1-specific neutralizing antibodies, which conferred a dose-dependent protection against lethal endotoxemia (23). In a more clinically relevant animal model of sepsis (induced by CLP), delayed administration of HMGB1-specific neutralizing antibodies beginning 24 h *after* the onset of sepsis, dose-dependently rescued mice from lethal sepsis (31,39).

HMGB1-mediated injurious inflammatory responses—Administration of exogenous HMGB1 to mice recapitulates many clinical signs of sepsis including fever (40), derangement of intestinal barrier function (41), and tissue injury (42–45). In the brain, exogenous HMGB1 induces the release of proinflammatory cytokines (46) and excitatory amino acids (such as glutamate) (47) and fever (40). In the lung, HMGB1 induces neutrophil infiltration and acute injury (42–45). Focal administration of HMGB1 near the sciatic nerve induces unilateral and bilateral low threshold mechanical allodynia (48). Similarly, intraperitoneal injection of HMGB1 induces peritoneal infiltration of neutrophils (49), and accumulation of cytokines (e.g., TNF and IL-6) and chemokines (e.g., MCP-1). Taken together, these experimental data establish extracellular HMGB1 as a critical late mediator of experimental sepsis, with a wider therapeutic window than early proinflammatory cytokines (Fig. 3).

HMGB1 as an early mediator of ischemic or traumatic injury

In contrast to the delayed systemic HMGB1 accumulation in experimental sepsis, HMGB1 functions as an early mediator in animal models of ischemia/reperfusion (I/R) injury (50–52). Similarly, HMGB1 release may be an early event in patients with hemorrhagic shock (53) or traumatic injury (54), because its circulating levels are elevated within 2–6 hours after onset of these diseases. Prophylactic administration of HMGB1-neutralizing antibody conferred protection against hepatic I/R injury in wild-type mice, but not in TLR4-defective (C3H/HeJ) mutant, implicating a role for TLR4 in HMGB1-mediated hepatic I/R injury (50). In contrast, treatment with HMGB1 antagonist (such as HMGB1 box A) significantly reduced myocardial ischemic injury in wild-type mice, but not in RAGE-deficient mutants, indicating a potential role for RAGE in HMGB1-mediated ischemic injury (55). The potential involvement of RAGE in HMGB1-mediated ischemic injury was further supported by the observation that genetic RAGE deficiency and the decoy soluble RAGE receptor similarly reduced cerebral ischemic injury (56).

In addition, HMGB1-specific neutralizing antibodies have been proven protective against ventilator-induced acute lung injury (57), severe acute pancreatitis (58), and hemorrhagic shock (53), supporting a pathogenic role for extracellular HMGB1 in various inflammatory diseases. Notably, HMGB1 is capable of attracting stem cells (59), and may be important for tissue repair and regeneration (1,60). For instance, although elevated serum HMGB1 levels were associated with adverse clinical outcomes in patients with myocardial infarction (61), prolonged blockade of HMGB1 with neutralizing antibodies (for 7 days) impaired healing process in animal models of myocardial ischemia/reperfusion. Therefore, like other cytokines, there may be protective advantages of extracellular HMGB1 when released at low amounts (60,62). It is thus important to pharmacologically modulate, rather than abrogate, systemic HMGB1 accumulation to facilitate resolution of potentially injurious inflammatory response.

EXTRACELLULAR HMGB1 AS AN ALARMIN SIGNAL

Recently, a number of ubiquitous, structurally and functionally diverse host proteins [such as HMGB1 and heat shock protein 72 (Hsp72)] have been categorized as “alarmins” based on the following shared properties (63) (Fig. 2).

1) Active release and passive leakage

As mentioned earlier, innate immune cells actively release HMGB1 in response to exogenous bacterial products (such as endotoxin or CpG-DNA) (23,64), or endogenous host stimuli (e.g., TNF, IFN- γ , or hydrogen peroxide) (23,65,66). Lacking a leader signal sequence, HMGB1 can not be actively secreted via the classical ER-Golgi secretory pathway (23). Instead, activated macrophages/monocytes acetylated HMGB1 at its nuclear localization sequences, leading to sequestration of HMGB1 within cytoplasmic vesicles and subsequent extracellular release (28,65,67). In addition, serine phosphorylation might be another requisite step for HMGB1 nucleocytoplasmic translocation (68). The phosphorylation of HMGB1 is potentially mediated by the Calcium/Calmodulin-Dependent Protein Kinase (CaMK) IV (69), because CaMK IV can be translocated to the nucleus following endotoxin stimulation, where it can potentially binds and phosphorylates HMGB1 (69). In addition, HMGB1 can be passively released from necrotic cells (70), or cells infected by viruses (e.g., West Nile, Salmon anemia, Dengue, and influenza viruses) (71–74) or mycobacteria (75,76), and similarly triggers inflammatory response (Fig. 3).

2). Stimulating cell migration

Accumulating evidence indicate that HMGB1 is capable of stimulating migration of neurite (77), smooth muscle cells (78), tumor cells (79), mesoangioblast stem cells (59,80), monocytes (81), dendritic cells (82,83), and neutrophils (49,84) (Fig. 3). It raises a possibility that extracellular HMGB1 may facilitate recruitment of innate immune cells to sites of infection or injury (85), thereby functioning as a potential host cell-derived chemotactic factor (86).

3). Facilitating innate recognition of microbial products

Recent studies suggested that HMGB1 can bind and facilitate innate recognition of bacterial products (e.g., CpG-DNA or LPS) by innate immune cells (such as macrophages and dendritic cells) (64,87,88). In addition, HMGB1 may also bind many endogenous molecules such as thrombomodulin (37), immunoglobulin (e.g., IgG1) (38), IL-1 (89), or nucleosomes derived from apoptotic cells (90). These different host factors have been shown to negatively (37) or positively (89,90) affect HMGB1-mediated inflammatory responses.

4). Activating innate immune cells

Accumulating evidence have suggested that extracellular HMGB1 binds to the receptor for advanced glycation end products (RAGE), and pattern-recognition receptors such as TLR2 and TLR4 (91,92). Consequently, HMGB1 activates innate immune cells (91–96) or endothelial cells (97,98) to produce proinflammatory cytokines, chemokines (95), and adhesion molecules (Fig. 3). *In vitro*, one of the DNA-binding domains of HMGB1, the “A box”, functions as an antagonist of HMGB1 (31,99,100). In contrast, another DNA-binding domain, the “B box”, recapitulates the cytokine activity of full length HMGB1 (101,102). Interestingly, oxidation of HMGB1 by reactive oxygen species (ROS) enables formation of disulfide bond between thiol group of Cys¹⁰⁶ and Cys²³ or Cys⁴⁵, and consequently abolish HMGB1-mediated immunostimulatory activities (103). Because Cys¹⁰⁶ is located within the 18-amino acid cytokine domain of HMGB1 B box, it will be important to investigate whether oxidization similarly affects HMGB1 cytokine activities in future studies.

5). Inhibiting phagocytotic elimination of apoptotic neutrophils

As mentioned earlier, macrophages recognize apoptotic cells through cell surface receptors for phosphatidylserine (PS). Interestingly, HMGB1 could interact with PS on cell surface of apoptotic neutrophils, and consequently inhibit phagocytotic elimination of apoptotic neutrophils by macrophages (Fig. 3) (104). Inefficient elimination of apoptotic cells may lead to excessive accumulation of late apoptotic and/or secondary necrotic cells, which may passively release proinflammatory mediators (such as HMGB1) (105). Considered together, these studies indicate that extracellular HMGB1 can function as an alarmin signal to recruit, alert and activate innate immune cells, thereby sustaining a potentially injurious inflammatory response.

POTENTIAL HMGB1-INHIBITING THERAPEUTIC AGENTS

With a limited number of effective therapies available for patients with sepsis, it is important to search for other agents capable of inhibiting clinically accessible late mediators. Below is a list of agents that have been proven protective against experimental sepsis partly through attenuating systemic HMGB1 accumulation (Table 1).

Antibodies

Anti-IFN-gamma antibodies—As mentioned earlier, pro-inflammatory cytokines (such as IFN- γ) effectively stimulate innate immune cells to actively release HMGB1 (65). In

animal model of sepsis, intravenous administration of IFN-gamma antibodies (1.2 mg/kg), immediately or 24 h after CLP, reduced peritoneal and serum HMGB1 levels, and attenuated CLP-induced animal mortality (106). It suggests that specific inhibition of HMGB1-stimulating proinflammatory cytokines may attenuate sepsis-induced HMGB1 accumulation, thereby protecting animals against lethal sepsis. In parallel with CLP-induced systemic HMGB1 accumulation, nucleocytoplasmic shuttling of HMGB1 occurs in alveolar macrophages at 24 h post CLP (107). The sepsis-induced HMGB1 translocation was associated with a significant decrease in TNF production (107), suggesting that HMGB1 deprivation is associated with macrophage suppression. The potential role for nuclear HMGB1 in the regulation of innate immune function (107) was further supported by the observation that HMGB1 binds to a cis-acting regulatory element (spanning from -157 to -137 bp of the 5'-flanking region) of the TNF gene to facilitate its transcription (96).

Intravenous immunoglobulin—Intravenous immunoglobulin (IVIG) refers to immunoglobulins (IgG, antibodies) pooled from the plasma of many healthy blood donors. It is usually given intravenously as a plasma protein replacement therapy to patients with various inflammatory diseases due to acute infections, autoimmune, or immune deficiencies. A recent study indicated that IVIG dose-dependently protected rats against sepsis-induced lung injury and lethality by attenuating systemic HMGB1 release (108). The mechanisms by which IVIG suppresses systemic HMGB1 release remains poorly understood. Notably, it has recently been found that human IgGs can bind to HMGB1, and potentially interfere with ELISA detection of HMGB1 (38). It is not known whether IVIG indeed attenuate systemic HMGB1 accumulation, or merely interfere with ELISA detection of HMGB1 (1).

Anti-coagulant agents

As a major regulator of hemostasis, thrombin is pro-coagulant by activating blood-clotting factors (Va, VIIIa and XI), cleaving fibrinogen to form a fibrin clot, and stimulating platelet aggregation. Its activities can be inhibited by various anti-coagulant factors such as anti-thrombin and thrombomodulin.

Anti-thrombin III—Antithrombin inhibits the pro-coagulant activities of thrombin upon interaction with heparin or related glycosaminoglycans. Although anti-thrombin III (AT-III) failed to reduce mortality rate in large sepsis clinical trial (109), a recent study suggested that AT-III could attenuate endotoxin-induced systemic HMGB1 accumulation, and reduced endotoxemic lethality (110). The mechanisms by which AT-III inhibits HMGB1 release remains to be further investigated.

Thrombomodulin—Thrombomodulin can bind thrombin to inhibit its pro-coagulant activities, and enhance its capacities to activate a plasma anticoagulant, activated protein C. Interestingly, human soluble thrombomodulin (ART-123) can physically bind to HMGB1 protein, thereby inhibiting HMGB1-mediated inflammatory response. Furthermore, ART-123 conferred significant protection against lethal endotoxemia (37), but it is not yet known whether ART-123 is protective in clinically relevant animal models of sepsis.

Endogenous hormones

Insulin—A recent study indicated that hyperglycemia, induced by infusion of glucose immediately following endotoxemia, aggravated endotoxin-induced HMGB1 release and lung injury (111). In contrast, intensive blood glucose control by insulin conferred protection against endotoxin-induced acute lung injury, and endotoxemic lethality (111). It is currently unknown whether the observed protective effects are dependent on insulin's anti-inflammatory activities, or its blood glucose-modulating properties (112).

Neuropeptides—Vasoactive intestinal peptide (VIP) is a short-lived small peptide hormone produced by the gut, pancreas and brain. It can induce smooth muscle relaxation, and is involved in communication between brain neurons. In animal models of sepsis induced by CLP, administration of VIP attenuated systemic HMGB1 accumulation, and consequently reduced animal lethality (113). Another member of the VIP family, the pituitary adenylate cyclase-activating polypeptide (PACAP), has also been shown protective against lethal endotoxemia partly by attenuating systemic HMGB1 accumulation (114). Another neuropeptide, urocortin, belongs to the corticotropin-releasing factor family. It is expressed in the brain, and may be responsible for regulation of appetite. It is also a potent and long-lasting hypotensive agent and increases coronary blood flow. In animal models of sepsis induced by CLP or bacteremia, administration of urocortin also attenuated systemic HMGB1 accumulation, and consequently reduced animal lethality (113), supporting a therapeutic potential for neuropeptides in the treatment of lethal systemic inflammatory diseases.

Ghrelin—Ghrelin is a stomach-derived hormone that is responsible for regulating the appetite – increasing it before eating and decreasing it afterward. Intriguingly, plasma ghrelin levels are significantly decreased in septic animals (115), and administration of ghrelin promoted a dose-dependent protection against sepsis-induced acute lung injury and lethality (115–117). Ghrelin may exert its protective effects through multiple mechanisms, such as by attenuating systemic HMGB1 release, and by facilitating bacterial elimination (117). Intriguingly, ghrelin may attenuate systemic accumulation of proinflammatory cytokines partly via the vagus nerve (116), suggesting that pharmacologic stimulation of the vagus nerve may be an effective therapy for experimental sepsis.

Vagus nerve stimulation

Recent evidence suggests that the central nervous system can attenuate peripheral innate immune response through efferent vagus nerve signals to tissue-resident macrophages (118). This effect is mediated by the principle neurotransmitter of the vagus nerve, acetylcholine, which inactivates macrophages via nicotinic cholinergic receptors (118). Indeed, stimulation of the vagus nerve by physical methods (e.g., electrical or mechanical) (119,120) or chemical agents (such as cholinergic agonists, nicotine, choline and GTS-21) (121–123) conferred protection against lethal endotoxemia and sepsis partly by attenuating systemic HMGB1 accumulation.

A chemical derivative of choline, stearyl lysophosphatidylcholine, has also been proven protective against experimental sepsis by stimulating neutrophils to destroy ingested bacteria in an H₂O₂-dependent mechanism (124). However, stearyl LPC also confers protection against lethal endotoxemia (124), implying that it may exert protective effects through an additional, bactericidal-independent mechanism (125). Indeed, administration of stearyl LPC significantly attenuated circulating HMGB1 levels (126), indicating that stearyl LPC protects against experimental sepsis partly by facilitating elimination of invading pathogens, and partly by attenuating systemic HMGB1 accumulation (125).

Chinese medicinal herbs

Traditional herbal medicine has formed the basis of folk remedies for various inflammatory ailments. Among several dozens commonly used Chinese herbs (127), we found that aqueous extracts of Danggui (*Angelica sinensis*), Green tea (*Camellia sinensis*), and Danshen (*Salvia miltiorrhiza*) efficiently inhibited endotoxin-induced HMGB1 release, and protected animals against experimental sepsis (128–130).

Danggui—Danggui has been traditionally used to treat gynecological disorders (such as abnormal menstruation). Its aqueous extract dose-dependently inhibited LPS-induced HMGB1 release in macrophage and monocyte cultures, partly by interfering with HMGB1 cytoplasmic translocation (128). Furthermore, Danggui extract rescued mice from lethal sepsis even when the first dose was given at 24 h post onset of disease (128). The active components responsible for these beneficial effects remain a subject of future investigation.

Green tea—Green tea brewed from the leaves of the plant, *Camellia sinensis*, contains a class of biologically active polyphenols called catechins. Accounting for 50–80% of the total catechin, EGCG, is effective in attenuating endotoxin-induced HMGB1 release by macrophage and monocytes (130). In addition, EGCG dose-dependently inhibited HMGB1-induced release of TNF, IL-6, and nitric oxide in macrophage cultures (130). Interestingly, EGCG completely abrogated accumulation/clustering of exogenous HMGB1 on macrophage cell surface (130), suggesting that EGCG inhibits HMGB1 cytokine activities by preventing its cell surface accumulation/clustering. *In vivo*, EGCG (10 mg/kg, intraperitoneally) improved animal survival in a rat model of CLP-induced experimental sepsis (131). Similarly, repeated administration of EGCG conferred a dose-dependent protection against lethal endotoxemia, and rescued mice from lethal sepsis even when the first dose of EGCG was given at +24 h after onset of sepsis (130). Consistently, delayed administration of EGCG significantly attenuated circulating levels of HMGB1, as well as surrogate markers of experimental sepsis (such as IL-6 and KC) (130,132). Considered together, these experimental data indicate that EGCG protects mice against lethal sepsis partly by attenuating systemic HMGB1 accumulation, and partly by inhibiting HMGB1-mediated inflammatory response.

Danshen—Another Chinese herb, Danshen has been widely used in China for patients with cardiovascular disorders (133,134). It contains abundant red pigments (termed tanshinone I, tanshinone IIA, and cryptotanshinone), which effectively attenuated LPS-induced HMGB1 release (129). A water-soluble derivative (sodium sulfonate) of tanshinone IIA, TSN IIA-SS, at concentrations (100 μ M) that completely abrogated LPS-induced HMGB1 release, only partially attenuated LPS-induced release of four out of 62 cytokines [e.g., IL-12p70, IL-1 α , platelet factor 4 (PF-4), and MCP-5] (129), indicating a specificity for TSN IIA-SS in inhibiting LPS-induced HMGB1 release. Despite a structural resemblance (i.e., the presence of a four-fused-ring structure) between tanshinones and steroidal anti-inflammatory drugs (such as dexamethasone and cortisone), tanshinones inhibit LPS-induced HMGB1-release in a glucocorticoid receptor-independent mechanism (129). More importantly, repeated administration of TSN IIA-SS 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 (129). Notably, administration of TSN IIA-SS dose-dependently attenuated circulating HMGB1 levels in septic mice (129), suggesting that TSN IIA-SS confer protection against experimental sepsis partly by inhibiting systemic HMGB1 accumulation.

Others

Ethyl pyruvate is an aliphatic ester derived from endogenous substance, pyruvic acid, which is a final product of glycolysis and the starting substrate for the tricarboxylic acid cycle (135). It dose-dependently inhibits LPS-induced release of early (e.g., TNF) and late proinflammatory cytokines (e.g., HMGB1), and protected mice against experimental sepsis, even when treatment is started as late as 12–24 hours after the onset of disease (136).

CONCLUSIONS AND PERSPECTIVES

For complex systemic inflammatory diseases such as sepsis, it appears difficult to translate successful animal studies into clinical applications (1). For instance, although neutralizing antibodies against endotoxin (22) or cytokines (e.g., TNF) (17,137) are protective in animal models of endotoxemia or bacteremia, these agents failed in sepsis clinical trials (138–140). This failure partly reflects the complexity of the underlying pathogenic mechanisms of sepsis, and the consequent heterogeneity of the patient population (3,141). It may also be attributable to pitfalls in the selection of: 1) feasible therapeutic targets or drugs; 2) optimal doses and timing of drugs; and 3) non-realistic clinical outcome measures (such as mortality rates) (3,141).

Intensive pre-clinical animal studies have established HMGB1 as a late mediator of experimental sepsis with a wider therapeutic window (142,143). 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 rescues mice from lethal experimental sepsis even when the first dose is given 24 hours after onset of sepsis. Will HMGB1 ever become a clinically feasible therapeutic target for human sepsis? This question can not be answered until HMGB1-inhibiting agents have been tested for efficacy in large clinical trials.

One of the most selective HMGB1 inhibitor, TSN IIA-SS, has already been used in China as a medicine for patients with cardiovascular disorders (133). Even in septic animals, TSN IIA-SS reduced total peripheral vascular resistance, and yet increased cardiac stroke volume and cardiac output (129). Because HMGB1 may function as a myocardial depressant factor by reducing contractility of cardiac myocytes (144), it is plausible that TSN IIA-SS improves cardiovascular function partly by attenuating HMGB1 release. The dual effects of TSN IIA-SS in attenuating late inflammatory response and improving cardiovascular function make it a promising therapeutic agent for sepsis. It is thus important to further investigate the intricate mechanisms by which various agents attenuate systemic HMGB1 release, and explore their therapeutic potential in future clinical studies.

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Fig. 1. A microbial infection can trigger a local or systemic inflammatory response

Upon disruption of epithelial barrier, microbial pathogens invade and elicit an innate immune response at infection site. If invading pathogens are effectively ingested and eliminated by neutrophils and macrophages, local inflammation resolves normally to regain immunological homeostasis. Otherwise, invading pathogens can leak into the blood stream, and trigger a potentially injurious systemic inflammatory response that could lead to sepsis.

Fig. 2. Early versus late mediators of septic lethality

Mice subjected to septic insult (such as cecal ligation and puncture, CLP) succumb at latencies of up to 1–3 days. Early cytokines (such as TNF) reach plateau levels in the circulation long before the onset of septic lethality (20–24 h post CLP) during an “early” stage of sepsis. In contrast, late mediators (such as HMGB1) reach peak levels in the circulation immediately after onset of septic lethality during a “late” stage of sepsis. This delayed systemic HMGB1 accumulation parallels with septic lethality, and provides HMGB1 with a wider therapeutic window.

Fig. 3. Extracellular HMGB1 functions as an alarmin signal

HMGB1 is actively secreted by innate immune cells in response to exogenous microbial products (e.g., LPS or CpG-DNA) or endogenous host stimuli (TNF, IFN- γ , or hydrogen peroxide), and passively released by damaged or virus-infected cells. Extracellular HMGB1 sustains an inflammatory response by stimulating migration of innate immune cells, facilitating innate recognition of bacterial products, activating various innate immune cells, and suppressing phagocytosis of apoptotic cells. Thus, HMGB1 can function as an alarmin signal to recruit, alert and activate various innate immune cells, thereby sustaining potentially injurious inflammatory response.

Table 1

Potential therapeutic agents for experimental sepsis.

Agents	Conferred Protection Against	By inhibiting HMGB1	References
Antibodies			
Anti-IFN- γ	Sepsis	release	106
Anti-HMGB1	Endotoxemia and Sepsis	activity	23, 31, 39
Intravenous immunoglobulin (IVIG)	Sepsis	release	108
Anti-coagulant agents			
Anti-thrombin III	Endotoxemia	release	110
Thrombomodulin	Endotoxemia	activity	37
Endogenous hormones			
Insulin	Endotoxemia	release	111, 112
Vasoactive intestinal peptide	Endotoxemia and Sepsis	release	113, 114
Ghrelin	Sepsis	release	115, 116, 117
Vagus nerve stimulation			
Electrical	Endotoxemia and Sepsis	release	118
Chemical (nicotine, GTS-21, choline)	Endotoxemia and Sepsis	release	119, 121, 122, 123
Mechanical (transcutaneous)	Endotoxemia and Sepsis	release	120
Chinese herbal components			
Danshen (TSN IIA-SS)	Endotoxemia and Sepsis	release	129
Green tea (EGCG)	Endotoxemia and Sepsis	release	130, 131
Others			
Ethyl pyruvate	Endotoxemia and Sepsis	release	135, 136
Stearoyl lysophosphatidylcholine	Endotoxemia and Sepsis	release	124, 126