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Targeting HMGB1 in inflammation

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Abstract

High mobility group box 1 (HMGB1), a highly conserved, ubiquitous protein present in the nuclei and cytoplasm of nearly all cell types, is a necessary and sufficient mediator of inflammation during sterile and infection-associated responses. Elevated levels of HMGB1 in serum and tissues occur during sterile tissue injury and during infection, and targeting HMGB1 with antibodies or specific antagonists is protective in established preclinical inflammatory disease models including lethal endotoxemia or sepsis, collagen-induced arthritis, and ischemia–reperfusion induced tissue injury. Future advances in this field will stem from understanding the biological basis for the success of targeting HMGB1 to therapeutic improvement in the treatment of inflammation, infection and ischemia–reperfusion induced injury.

Keywords

Cytokine; HMGB1; Inflammation; Immune response; Receptor

1. Introduction

Immunity, the ability to resist invasion, can be either inherited (innate) or acquired (adaptive). Cells of the innate immune system, including monocytes, macrophages, and neutrophils are on the front line in the host response to infection, invasion, and injury. During infection, innate immunity is activated by foreign molecular products, termed pathogen associated molecular patterns (PAMPs) including lipopolysaccharide (LPS), double stranded RNA, CpG DNA, and enterotoxins. During sterile injury or ischemia, these same cells are activated by exposure to damage associated molecular pattern (DAMPs), including heat shock proteins, uric acid, annexins, and IL-1alpha. Activation of innate immunity initiates the production and release of cytokines, proteins that mediate diverse metabolic and immunological responses in other cells [1]. Cytokine release is necessary and sufficient to initiate inflammation, the syndrome of pain, swelling, hyperemia, and hyperthermia that heralds the onset of both infection and sterile injury. The magnitude of the cytokine response is tightly regulated, because the over-production of cytokines can directly mediate the pathogenesis of inflammatory diseases, and if severe enough, lethal shock and tissue injury [2]. The “cytokine theory of disease,” which is the paradigm that cytokines are both necessary and sufficient to cause disease, has been validated by the clinical success of

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selectively targeting individual cytokines to therapeutic advantage [3]. For example, anti-TNF antibodies are effective in the treatment of rheumatoid arthritis, inflammatory bowel disease, and psoriasis, and strategies targeting IL-1 and Il-6 are also widely used [4,5].

Despite these advances, not all patients respond to currently available cytokine based therapeutics; and additional experimental therapeutic approaches are necessary, and being pursued. Accordingly, with our colleagues in the early 1990s, we initiated a search for previously unrecognized cytokine mediators of inflammation released by exposing macrophages to the prototypical PAMP, Gram-negative lipopolysaccharide. This effort culminated in the isolation and identification of HMGB1 as an inducible macrophage secreted protein that mediated endotoxin lethality and activated innate immune cells to produce cytokines [6,7]. The discovery that a ubiquitous, 30 KDa nuclear DNA-binding protein had roles not only in stabilizing DNA structure and mediating neurite outgrowth, but also in mediating and modulating the inflammatory response to invasion, enabled development of numerous strategies to selectively neutralize the activity of HMGB1 with antibodies or other agents which suppress inflammation in standardized preclinical studies. Not surprisingly, like other inducible inflammatory mediators, the activities of HMGB1 are synergistically increased by interaction with LPS, CpG DNA, IL-1, and other cytokines [8].

An important question in the biology of inflammation is how can infectious and sterile inflammation initiate nearly identical physiological responses? The answer came from the discovery by Billiar et al. that in addition to being a therapeutic target for diseases initiated by PAMPs, HMGB1 is also necessary for the pathogenesis of ischemia and sterile, non-invasive inflammation [9–11]. Ischemia and cell damage lead to the passive release of endogenous HMGB1, which in turn functions to mediate cytokine release, inflammation, and tissue injury by activating innate immunity through signal transduction in Toll-like receptors (TLRs) and the receptor for advanced glycation end products (RAGE). HMGB1 thus mediates inflammation by activating innate immune receptors during sterile injury, which are comparable to activation by PAMPs. Here we focus on reviewing the pathogenic role of HMGB1 in sterile and infection-induced inflammation, its activities in preclinical disease models, and methods to modulate its activity to therapeutic advantage.

2. Cytokine activity of HMGB1

HMGB1 can be actively released from immune cells including macrophages, monocytes, NK cells, dendritic cells, endothelial cells, and platelets [6,7,12,13]. It is also passively released from necrotic or damaged cells [10,14–16]. Both mechanisms can produce the release of significant amounts of extracellular HMGB1. Although apoptotic cells release significantly less HMGB1 as compared to necrotic cells, macrophage engulfment of apoptotic cells leads to significant HMGB1 release [17–19]. Administration of HMGB1 to normal animals produces systemic inflammatory responses including fever, weight loss and anorexia, acute lung injury, epithelial barrier dysfunction, arthritis and death [16]. Anti-HMGB1 treatment, with either antibodies, specific antagonists or other pharmacological agents, is beneficial in many preclinical inflammatory diseases models, ameliorates severity of diseases and reduces mortality (summarized in Table 1 and illustrated in Fig. 1).

The inflammatory activity of HMGB1 is dependent upon the oxidation status of cysteine 106 residing within the B box DNA-binding domain of HMGB1, a region that is critical for stimulating cytokine release and inflammation [20–22]. Recent studies have revealed that cysteine 106 is critically important for HMGB1 binding to TLR4/MD2, as revealed by using HMGB1 protein with a point mutation at 106 cysteine (replaced by alanine) and by using synthetic 20-mer peptide covering 106 cysteine (our unpublished data). Together, these results indicate that the cysteine 106 is required for HMGB1 signaling through TLR4 to stimulate cytokine release and inflammation.

3. Receptors mediating HMGB1 activity

Once released into the extracellular milieu, HMGB1 can bind to cell surface receptors including the RAGE, TLR2, TLR4, and TLR9 [8,16,23]. HMGB1 interaction with these receptors transduces intracellular signals and mediates cellular responses including chemotactic cell movement and release of pro-inflammatory cytokines (e.g., TNF and IL-1) *in vitro*; and causes fever, epithelia barrier dysfunction, and acute inflammation *in vivo*.

3.1. In vivo

3.1.1. RAGE—RAGE is a transmembrane protein and a member of the immunoglobulin superfamily. RAGE is expressed in endothelial cells, vascular smooth muscle cells, neurons and macrophages/monocytes [24]. *In vivo* experiments indicate that HMGB1/RAGE interaction may be important in tumor formation and proliferation, because blocking RAGE and HMGB1 can decrease tumor growth and metastasis in mice [25].

Anti-RAGE antibodies reduced HMGB1 expression in the diagram during sepsis induced by cecal perforation. Neutralizing RAGE and HMGB1 by antibodies attenuated diaphragm dysfunction in septic rats induced by cecal perforation [26].

3.1.2. TLR4—Toll-like receptor 4 (TLR4) has been implicated as the critical receptor mediating the inflammatory activity of HMGB1. HMGB1 is a mediator in ischemia and reperfusion damage in liver, heart, and kidney [10,15,27,28]. Ischemia followed by reperfusion leads to severe organ injury and dysfunction. Reperfusion activates innate immune responses and induces cytokine release which mediates the development of systemic inflammatory responses and further tissue damage. Tsung et al. showed that hepatic HMGB1 levels are increased rapidly during ischemia–reperfusion injury, rise within 1 h after reperfusion, and remained elevated for up to 24 h in animal models [10]. Anti-HMGB1 antibodies failed to provide protection in TLR4 defective C3H/HeJ mice, but successfully reduced damage in C3H/OuJ mice, indicating that TLR4 is involved in this HMGB1 mediated hepatic injury [10]. Mechanistically, HMGB1 release during liver ischemia–reperfusion involves TLR4-dependent production of reactive oxygen species and calcium-mediated signaling [27]. In agreement with these observations, TLR4 is implicated as a receptor mediating ischemia–reperfusion disorders in the kidney and cold ischemia–reperfusion of the heart [28–30].

3.2. In vitro

3.2.1. RAGE—HMGB1 binds to RAGE in a concentration-dependent manner [31]. 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 [8,16,32,33]. A structure/functional analysis revealed that amino acids 150–183 in the C terminus of HMGB1 are responsible for RAGE binding [34]. Anti-RAGE antibodies partially inhibit HMGB1-induced chemokine and cytokine release in endothelial cells [35]. Hence, HMGB1 interacts with RAGE, but interaction with TLR2 and TLR4 are required for HMGB1 signaling in macrophages as described below.

3.2.2. 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 [10,27,36–38]. 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 [39]. HMGB1 signals via TLR4 to activate tumor antigen-specific T cell immunity in both mice and humans [36]. *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 [37].

3.2.3. 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 [40]. In human embryonic kidney (HEK293) cells transfected with TLR2, TLR4, or vector alone, HMGB1 effectively induces IL-8 release only from TLR2 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 [39]. 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 [41].

4. HMGB1 links sterile injury and infection-induced immunity

The discovery of the cytokine role of HMGB1 has led to a new understanding of sterile and infection-induced inflammation. HMGB1 produced by cell injury activates innate immunity, and this response is qualitatively indistinguishable from the response activated from infectious insult. HMGB1 activates these responses by signaling through the same receptor family that is activated by exogenous agents. Low levels of HMGB1 are present in the serum of healthy subjects (10–30 ng/ml) [42] and in unstimulated cell culture supernatant. These levels do not cause inflammation. Large amounts of HMGB1 produced during severe tissue injury or cell death (i.e. crush injury) are inflammatory and associated with fever, anorexia, epithelial leakage syndrome and organ failure [8,16]. HMGB1 derived from

Chinese hamster ovary mammalian cells genetically engineered to continuously secrete HMGB1 stimulates cytokine release from human macrophages, and neutralizing monoclonal anti-HMGB1 antibodies inhibit this activity [43]. Further, small amounts of HMGB1 that are ubiquitously present in the extracellular milieu synergistically enhance the inflammatory response induced by bacterial products. For example, binding of HMGB1 to LPS, CpG DNA, viral RNA and IL-1 all significantly increase the inflammatory activity of HMGB1, which can be inhibited with anti-HMGB1 antibodies [8,44]. The release of large quantities of HMGB1 during endotoxemia or infection can be lethal [6,21].

5. HMGB1 in diseases and studies targeting HMGB1 in the treatment of preclinical diseases

Treatment with inhibitors of HMGB1 activity is beneficial and reduces inflammation in dozens of preclinical animal studies. Here we discuss the therapeutic strategies that have been proven effective by inhibiting HMGB1 activity in a wide range of preclinical disease models (Table 1 and Fig. 1). We focus on studies using anti-HMGB1 antibodies or specific HMGB1 antagonist A box. Other pharmacological agents that act by targeting HMGB1 are also discussed.

HMGB1 A box is one of the DNA-binding motifs of HMGB1. Originally, A box was described as participating in maintaining nucleosome structure and regulation of gene transcription [45]. We made several deletion mutants of HMGB1 and found that A box is a specific HMGB1 antagonist [21]. *In vitro* studies showed that A box competitively inhibits ^{125}I-HMGB1 surface binding on macrophages and attenuates HMGB1-induced pro-inflammatory cytokine release in macrophage-like RAW 264.7 cells [21]. Since this was first described in 2004, many articles have shown that treatment with HMGB1 antagonist A box inhibits HMGB1 activity and is beneficial in inflammatory disease models as listed below and in Table 1. Other pharmacological agents inhibit HMGB1 activities, as summarized below.

5.1. Endotoxemia

LPS administration is a well established shock model used to study cytokine responses in animals and humans [16,23]. In this model, serum cytokine levels such as TNF and IL-1 rise rapidly (within 2 h) after administration of LPS [23]. The kinetics of HMGB1 release is delayed compared to other early cytokines. This delayed HMGB1 response is different from TNF and distinguishes HMGB1 from other early-acting pro-inflammatory cytokines [16,46]. Serum HMGB1 levels remained unchanged during the first 8 h after injection of an LD_{50} dose of LPS in mice, increased significantly after 16 h and stayed at elevated plateau levels for at least 36 h [6]. Passive immunization with polyclonal anti-HMGB1 antibodies significantly protects against lethal endotoxemia in mice, even when treatment was delayed 2 h after LPS exposure [6,47]. The effects of anti-HMGB1 antibodies were dose-dependent and were effective even after the peak of circulating TNF was resolved [6]. Similar protective effects were observed with treatment of HMGB1 A box in endotoxemia model in mice [21].

One of the modes for active HMGB1 release is to migrate from nuclei to cytoplasm and then released into extracellular milieu [16,48,49]. A variety of pharmacological agents that inhibit the nuclear release of HMGB1 from macrophages have been used in the treatment of inflammatory diseases. Reduced nuclear export of HMGB1 release using vagus nerve stimulation through alpha-7 nicotinic improved survival and ameliorated end organ dysfunction in endotoxemia in mice [50–52].

Thrombomodulin is an endothelial anticoagulant cofactor that promotes thrombin-mediated formation of activated protein C. Thrombomodulin binds to HMGB1 via N terminal lectin domain of thrombomodulin [47]. Administration of recombinant soluble thrombomodulin neutralizes HMGB1 activity and improves survival in endotoxemia [47,53].

5.2. Sepsis induced by cecal perforation

Sepsis is a syndrome mediated by the systemic inflammatory responses to infection. Severe sepsis is sepsis with evidence of organ dysfunction, such as hypoxemia, oliguria, lactic acidosis, or altered cerebral function [54]. Cecal ligation and puncture (CLP) is a standard model of murine sepsis in which the cecum is ligated and punctured to induce peritonitis and sepsis. Serum HMGB1 levels are elevated in this model of sepsis in a delayed pattern [21]. Serum HMGB1 levels are also significantly increased in sepsis patients, HMGB1 levels are higher in patients who succumbed to the disease than in survivors [6]. Sustained elevated serum HMGB1 levels are observed in patients with community-acquired pneumonia, the most common cause of severe sepsis; and higher levels of circulating HMGB1 are associated with mortality [42].

The protection conferred by anti-HMGB1 antibodies in endotoxemia is also observed in CLP sepsis [21,55]. Delayed treatment with either anti-HMGB1 antibodies or A box dose-dependently rescued mice from sepsis, and treatment was effective even when the first dose was given at 24 h after cecal ligation and puncture [21]. Recently developed anti-HMGB1 monoclonal antibodies also confirmed these findings [17]. This effective treatment by delayed administration of HMGB1 antibodies or antagonist A box in sepsis demonstrates a wider window of opportunities as composed to early pro-inflammatory cytokines.

Blockade of RAGE–HMGB1 signaling by treatment with monoclonal anti-RAGE antibodies increases survival in septic rats [56]. Importantly, anti-TNF is ineffective in this model, indicating that HMGB1, not TNF is a necessary mediator of lethal sepsis.

Green tea extract, Chinese medicinal herbal Danshen, vagus nerve stimulation, cisplatin (an anti-tumor drug that induces DNA lesions) and ethyl pyruvate (a non-toxic food additive and an experimental anti-inflammatory agent) all inhibit HMGB1 release [51,52,57–63].

HMGB1 binds LPS in a dose-dependent manner as revealed by surface plasmon resonance (BiaCore) and this binding enhances LPS-mediated TNF production in human monocytes [64]. Polymyxin B, which binds LPS, also binds and removes HMGB1 from circulation. Hemoperfusion treatment with polymyxin B-immobilized filter column effectively removes HMGB1 from circulation in piglets subjected to cecal perforation and reduces serum HMGB1 levels in patients with septic shock [65,66].

5.3. Gastro-intestinal disorders

HMGB1 impairs intestinal barrier function by increasing both ileal mucosa permeability and bacterial translocation to mesenteric lymph nodes [67]. A fundamental mechanism of HMGB1-mediated toxicity and lethality *in vivo* is attributable to epithelial dysfunction and leakage [67,68]. In inflammatory bowel disease induced by dextran sulfate sodium salt, treatment with polyclonal anti-HMGB1 antibodies reduced inflammation and reduces tumor incidence in colitis-associated cancer [69]. Anti-HMGB1 antibodies also reversed LPS-induced gut barrier dysfunction (increased permeability and bacterial translocation) in rats [70]. Blockade of RAGE–HMGB1 signaling by treatment with monoclonal anti-RAGE antibodies ameliorates intestinal barrier function after hemorrhagic shock and resuscitation [71].

5.4. Pancreatitis

Serum HMGB1 levels are significantly elevated in patients with acute pancreatitis, and the levels correlate with severity. Similar findings have been observed in animal models of pancreatitis [72,73]. Treatment with anti-HMGB1 antibodies significantly reduced the incidence of multiple organ failure, and increased survival [74]. In severe acute pancreatitis, induced by 20% L-arginine injection, A box treatment lowered serum HMGB1 levels, had protective effects against organ injury, and improved survival [75]. Ethyl pyruvate, by reducing nuclear HMGB1 release, also showed protective effects against organ damage in severe acute pancreatitis in rodents [76–78].

5.5. Respiratory disorders

Elevated HMGB1 levels are observed in plasma and lung epithelial lining fluid of patients with acute lung injury and in mice instilled with LPS [79,80]. Addition of HMGB1 intra-tracheally in mice causes acute lung injury as manifested by neutrophil accumulation, lung edema and increased pulmonary cytokine levels including TNF, IL-1β and MIP-2 [79]. Blockade of HMGB1 by polyclonal anti-HMGB1 antibodies ameliorated LPS-induced acute lung injury and inflammation, reduced ventilator-induced lung injury, prevented hemorrhage-induced pulmonary levels of pro-inflammatory cytokines and accumulation of neutrophils, and suppressed the development of pulmonary fibrosis in a mouse model of bleomycin-induced lung injury [79–83]. Treatment with A box conferred protection against lung injury in mice by reducing neutrophil filtration and decreasing the expression of chemokines and pro-inflammatory cytokines including HMGB1 [84]. Hemoperfusion treatment with polymyxin B-immobilized filter column effectively reduces serum HMGB1 levels in patients with acute respiratory distress syndrome 9 characterized by inflammation and increased permeability edema in the lung [85]. Administration of recombinant soluble thrombomodulin neutralizes HMGB1 activity and ameliorates injury-induced lung thrombosis and inflammation [86].

5.6. Arthritis

Rheumatoid arthritis is a chronic, systemic, autoimmune and inflammatory disorder that affects the synovial lining of the joints. Inhibiting HMGB1 activity is therapeutic in arthritis, because administration of either anti-HMGB1 or A box in collagen type II-induced arthritis

significantly attenuated the severity of disease [87,88]. HMGB1 levels in both serum and synovial fluid are significantly elevated in patients with rheumatoid arthritis [88–91]. Recent studies suggest that tissue hypoxia and extracellular HMGB1 release play an important role in the pathogenesis of arthritis [88]. Immuno-staining of synovial tissues from collagen type II induced-induced arthritis showed that HMGB1 is abundantly expressed as a nuclear, cytoplasmic and extracellular component, compared with specimens from normal rats, in which HMGB1 is primarily confined to the nucleus [90]. Intra-articular administration of HMGB1 induces the onset of arthritis in mice suggesting the important role of HMGB1 in the pathogenesis of arthritis [87,92]. Blockade of RAGE–HMGB1 signaling by treatment with monoclonal anti-RAGE antibodies reduced the severity of arthritis [93]. Pharmacological agents that reduce nuclear export of HMGB1 release have been shown to attenuate arthritis and inflammation in collagen-induced arthritis, by using oxaliplatin (gold sodium thiomalate, a traditional therapy for arthritis) and pituitary adenylate cyclase-activating polypeptide and by stimulation of nicotinic acetylcholine receptor [94–96]. Also, administration of recombinant soluble thrombomodulin neutralizes HMGB1 activity and protected against arthritis [97].

5.7. Hemorrhagic shock

Hemorrhagic shock, in the absence of infection, induced an increase in serum HMGB1 levels within hours after aortic aneurysm rupture in a human patient [98]. In animal studies, HMGB1 is released early during the course of hemorrhagic shock, and HMGB1 plays a role in the pathogenesis of hemorrhagic shock-induced tissue damage [38,71,81,99]. Treatment with anti-HMGB1 antibodies improves survival and reduces hemorrhage-induced acute lung injury, gut barrier dysfunction [71,81,99]. Mechanistically, hemorrhagic shock-induced lung injury and NADPH oxidase activation in neutrophils are HMGB1-TLR4 mediated events [38,100].

5.8. Stroke

Ischemic injury in the central nervous system (stroke) is mediated by glutamate excitotoxicity-induced neuron death. In ischemic stroke, the necrotic core is surrounded by a zone of inflammation, in which delayed cell death aggravates the initial insult. HMGB1 has been shown as a mediator in both the initial and delayed injury processes [101]. Patients with stroke had elevated serum HMGB1 levels hours after the onset of symptoms [102]. Tissue ischemia in animal models causes HMGB1 release into the extracellular milieu [103–105]. Nuclear HMGB1 translocates into a cytoplasmic region of neurons within 1 h of cerebral artery occlusion and HMGB1 gets fully depleted during the excitotoxicity-induced acute process [106]. Within 2 days after reperfusion, HMGB1 is expressed in activated microglia cells, astrocytes, macrophages and endothelial cells in the penumbra [106]. Elevated extracellular HMGB1 levels stimulate transporter-mediated glutamate release and in turn mediate cytotoxicity [107,108]. HMGB1 release can activate astrocytes and microglia, which are the markers of brain inflammation. HMGB1 also activates macrophages/monocytes to release the pro-inflammatory cytokines TNF and IL-6, and enhances pro-coagulant activity which contributes to tissue damage by clotting the micro-vasculature and worsening ischemia in the penumbra region [108,109]. Intra-cerebroventricular injection of recombinant HMGB1 worsens the severity of tissue damage

induced by infarction. Administration of both polyclonal and monoclonal anti-HMGB1 antibodies to rats subjected to middle cerebra artery occlusion had significant protective effects and reduced cell death [110,111]. Administration of A box ameliorated brain damage in a similar pattern as treatment with anti-HMGB1 antibodies [111].

5.9. Ischemia–reperfusion injury

Ischemia–reperfusion induces cytokine-driven inflammatory responses and tissue injury. Treatment with polyclonal anti-HMGB1 antibodies attenuates hepatic injury during liver ischemia–reperfusion [10]. HMGB1 acts as an early mediator of inflammation and organ damage in ischemia–reperfusion injury of the heart [15]. Treatment with A box significantly reduced infarct size and markers of tissue damage whereas administration of HMGB1 worsens the injury [15].

5.10. Transplantation

HMGB1 levels are elevated and reflect the extent of hepatocellular injury in human liver transplantation [112]. In animal models, HMGB1 plays a pivotal role in acute allograft rejection in murine cardiac transplantation [113]. Therapeutic administration of A box significantly enhances cardiac allograft survival and is associated with reduced allograft expression of TNF, IFN-gamma and HMGB1 [113].

6. Conclusion: targeting HMGB1 in inflammatory diseases

The original suggestion to target the inflammatory activity of HMGB1 to confer protection against tissue injury has been validated in dozens of preclinical studies. As both a secreted effector molecule produced during PAMP-activation of innate immunity, and an inflammatory mediator produced during sterile injury as a DAMP-signal, HMGB1 occupies a crucial signaling role at the intersection of these pathways. Ongoing work into the nature of HMGB1-receptor signal transduction, and its interaction with other mediators associated with activation of cytokine networks, will continue to expand biological understanding. The discovery of the significant protection afforded by anti-HMGB1 antibodies in diverse preclinical studies of innate immunity provides a new paradigm for strategic development of experimental therapeutics.

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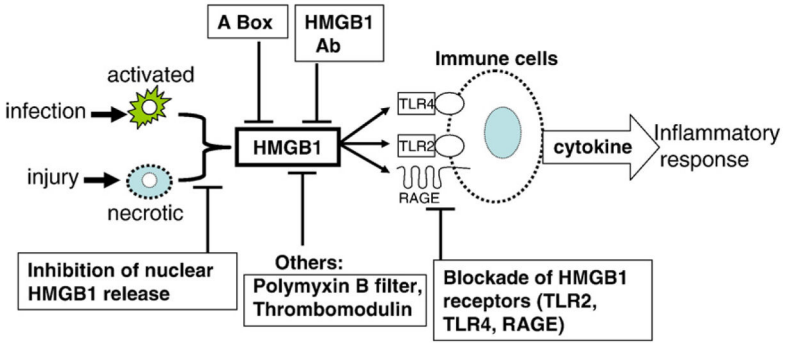

Fig. 1.

Strategies targeting HMGB1 in inflammatory diseases. HMGB1 can be actively secreted by innate immune cells in response to exogenous microbial products from infection; or passively released from injured or necrotic cells. 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 treatment is beneficial in many preclinical disease models as described in the text by using anti-HMGB1 antibodies, specific HMGB1 antagonist A box, blockade of HMGB1 receptors, or other pharmacological agents partially through targeting HMGB1.

Table 1

Therapies targeting HMGB1 in preclinical disease models.

Mode of anti-HMGB1	Disease model, effects, and species	References
Anti-HMGB1 antibodies	Endotoxemia: polyclonal anti-HMGB1 antibodies (pAb) treatment improved survival in mice.	[6,47]
	Sepsis induced by cecal perforation: both pAb and monoclonal antibody (mAb) treatment improved survival in mice.	[17,21,55]
	Gastro-intestinal disorders: pAb reversed LPS-induced gut barrier dysfunction in rats; reduced inflammation in murine colitis.	[69,70]
	Pancreatitis: pAb attenuated inflammation in acute pancreatitis in mice.	[74]
	Respiratory disorders: pAb ameliorated LPS-induced acute lung injury; ventilator-induced lung injury; pulmonary fibrosis in mice.	[79–83]
	Arthritis: pAb treatment attenuated arthritis and inflammation in collagen-induced arthritis in rodents.	[87–88]
	Hemorrhagic shock (HS): pAb improved survival and lung function after HS in mice.	[81,99]
	Stroke: MAb or pAb treatment ameliorated brain infarction in rats.	[110,111]
	Ischemia–reperfusion injury: pAb ameliorated hepatic ischemia–reperfusion injury in mice.	[10]
HMGB1 A box	Endotoxemia and sepsis induced by cecal perforation in mice: A box treatment improved survival in these models.	[21]
	Pancreatitis: A box protected organ damage caused by pancreatitis in mice.	[75]
	Respiratory disorders. A box reduced LPS-induced lung injury in mice.	[84]
	Arthritis. A box attenuated collagen-induced arthritis in rodents.	[87]
	Stroke: A box ameliorated ischemia brain damage.	[111]
	Ischemia–reperfusion injury: A box reduced damage in ischemia–reperfusion injury of heart in mice.	[15]
	Transplantation. A box prolonged cardiac allograft survival in rodents.	[113]
Others: blockade of RAGE–HMGB1 signaling	Sepsis induced by cecal perforation: improved survival in mice.	[26,56]
	Gastro-intestinal disorders: ameliorated intestinal barrier function after hemorrhagic shock and resuscitation in mice.	[71]
	Arthritis: reduced severity of arthritis in rodents.	[93]
Reduce nuclear HMGB1 release	Endotoxemia: improved survival, reduced end organ damage in mice.	[51,52,114,115]
	Sepsis induced by cecal perforation: improved survival in mice.	[51,52,57–63]
	Pancreatitis: attenuated organ damage in rodents.	[76–78]
	Arthritis: attenuated arthritis and inflammation in collagen-induced arthritis in rodents.	[94–96]
Polymyxin B filter	Sepsis: improved outcome in sepsis patients and piglets by removing HMGB1 from circulation.	[65,66]
	Respiratory disorder: reduced inflammation in patients with acute respiratory distress syndrome.	[85]
Thrombomodulin	Endotoxemia: improved survival in rodents.	[47]
	Respiratory disorder: ameliorated injury-induced lung injury in mice.	[53,86]
	Arthritis: protected against arthritis in mice.	[97]