

HIGH MOBILITY GROUP BOX 1 PROTEIN AS A LATE-ACTING MEDIATOR OF ACUTE LUNG INFLAMMATION

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Abstract. Acute inflammatory lung injury is often a delayed complication of critical illness and is associated with increased mortality. High mobility group box 1 (HMGB1) protein, in addition to its role as a transcriptional regulator factor, has been identified as a late mediator of endotoxin lethality and might be also involved in the development and progression of acute lung injury. HMGB1 protein itself can cause an acute inflammatory response manifested by increased production of proinflammatory cytokines and neutrophil accumulation. The delayed kinetics of HMGB1 protein release indicate that this protein is a distal mediator of acute inflammatory lung injury. Anti-HMGB1 protein antibodies attenuated endotoxin-induced lung injury, but not the early release of TNF- α and IL-1 β , indicating that HMGB1 protein is a late mediator of endotoxin-induced acute lung injury. HMGB1 protein is not released by apoptotic cells but is passively released by necrotic or damaged somatic and immune cells and it functions as a major stimulus of necrosis-induced inflammation. HMGB1 protein is also released by activated monocytes/macrophages and induces delayed and biphasic release of proinflammatory mediators from these cells. HMGB1 protein failed to stimulate cytokines release in lymphocytes, indicating that cellular stimulation is specific. We would like to suggest that HMGB1 protein may be also a primary mediator of the inflammatory responses to lung cells injury caused by toxic environmental chemicals.

Key words:

HMGB1 protein, lung inflammation, endotoxemia, macrophages, cytokines

INTRODUCTION

Acute lung injury develops in patients of all ages from variety of clinical disorders, including sepsis (pulmonary and non-pulmonary), pneumonia (bacterial, viral and fungal), aspiration of gastric and oropharyngeal contents, major trauma, and several other clinical disorders, including severe acute pancreatitis, drug overdose, and blood products [1,2]. Diffuse injury and infection of the lung are major causes of systemic inflammatory response, and dysregulation of cellular responses in the lung may result in circulating cytokines and other inflammatory and thrombotic

mediators. Signaling pathways, such as those provided by cytokines and their receptors, may have protective or injurious effects depending on the site of challenge and whether the inflammation is local or systemic [3,4]. Pro-inflammatory cytokines, tumor necrosis factor α (TNF- α) and interleukine 1 β (IL-1 β) are early mediators of lung inflammation and are produced by monocyte/macrophages in response to bacterial products, including endotoxins (lipopolisaccharide, LPS). The magnitude of the TNF- α response influences the development of shock, tissue injury, and death [5]. Inhibiting TNF- α activity or synthesis with

* Received: February 20, 2004. Accepted: March 26, 2004.

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specific antibodies prevents the development of lethal sepsis or endotoxemia [6,7]. This and another observation [8] that infusion of TNF- α to otherwise healthy mammals induces a disease indistinguishable from endotoxemia, demonstrate that TNF- α itself is sufficient to mediate lethal systemic inflammatory disease. TNF- α is released from monocyte/macrophages, neutrophils and other cells within the first few hours of the inflammatory response [9]. Endotoxin infusion to animals induces a rapid and transient release of TNF- α (and IL-1 β), which peaks and returns to basal levels within hours [6]. It was interesting that animals frequently succumb to systemic inflammatory responses at latencies of up to 5 days. Animals which are deficient in TNF- α die several days after exposure to lethal doses of endotoxin [10]. These suggest that downstream effectors can contribute to the pathology of endotoxemia late after disease onset. Wang et al. [11] identified high mobility group box 1 (HMGB1) protein as a late mediator of lethal inflammation. Blood serum HMGB1 levels are first detectable 12-16 h after endotoxin infusion and then remain increased for a prolonged period that coincides with lethality. Anti-HMGB1 antibodies rescue animals from endotoxemia and from established lethal systemic inflammation even when administered as late as 24 h after disease onset. Because endotoxin and proinflammatory cytokine release is also important in the mediation of acute lung injury, Abraham et al. [12] suggest that HMGB1 protein might also be involved in the development and progression of this entity. These authors show that HMGB1 protein itself can cause acute pulmonary inflammatory response, manifested by neutrophil accumulation, interstitial edema, and increased production of pro-inflammatory cytokines in the lungs. Anti-HMGB1 protein antibodies attenuated endotoxin-induced lung injury, but not the early release of TNF- α and IL-1 β [13], indicating that HMGB1 protein is a late mediator of endotoxin-induced acute lung injury. A series of recent discoveries revealed a cytokine activity of HMGB1 protein that when secreted into the extracellular milieu induces not only acute lung injury but also lethal systemic inflammatory responses, intestinal barrier dysfunction, and arthritis [14-20].

MOLECULAR STRUCTURE AND CELLULAR FUNCTIONS OF HMGB1

High mobility group box 1 protein is a member of the superfamily of high mobility group proteins (named for its electrophoretic mobility), discovered in the 1970s as an abundant chromosomal protein [21]. The high mobility group proteins are now classified in 3 groups on the basis of their structure: the HMGB family, which is characterized by a functional domain known as HMG "box"; the HMGN family, which contains a characteristic nucleosomal binding domain; and the HMGA family, which contains a unique "AT-hook" domain [22]. HMGB family members are among the most evolutionary conserved proteins, with 80% amino acids sequence identity among higher eukaryotic species [23]. Human HMGB1 protein is a single-chain protein and has 219 residues in its primary amino acid sequence. This protein is devoid of any enzymatic activity and has a molecular mass ~27 kilodaltons (kDa). The structural hallmarks of HMGB1 protein are two tandem L-shaped domains, named HMG-box A (in the N-terminal of polypeptide chain) and HMG-box B (in the central fragment of polypeptide chain), and a 30 amino acid-long, highly acidic COOH-terminal "tail". The N-terminus is rich in positively charged lysine residues, whereas C-terminus is rich in negatively charged aspartic and glutamic acid residues. The tandem HMG boxes and the C-terminal acidic tail are binding to DNA [24,25].

HMGB1 protein is an abundant component of chromatin as each nucleus contains some of its 10^6 molecules, that is, one molecule per 10 nucleosomes. HMGB1 protein molecules are in constant rapid motion. This means that the residence time of HMGB1 protein in any nuclear structure, such as chromatin, is very short. It is likely that the high intranuclear mobility of HMGB1 protein leads to frequent collisions with the chromatin fibre and promotes interactions with other nuclear proteins [26]. Specific nuclear targets for HMGB1 have not been identified, yet the protein facilitates numerous nuclear functions, including transcription, replication, and recombination as well as the action of p53, steroid hormone receptors, glucocorti-

coid receptor, and homeobox proteins [27,28]. The ability of HMGB1 protein to affect many types of nuclear activities reflects its mode of binding to DNA and its ability to interact with a diverse set of proteins. All these modes of action depend on the ability of HMGB1 protein to move rapidly throughout the nucleus and thus it may be an essential element able to participate in diverse nuclear function [29,30].

Post-translational modification of HMGB1 protein by co-activators is also emerging as an important mechanism in transcriptional processes. HMGB1 is subject to extensive post-translational modification, including glycosylation, phosphorylation, and acetylation, but native HMGB1 protein has not been isolated from pathologic material, and it is not known whether the "pathologic" protein differs significantly from HMGB1 found in normal conditions. Native HMGB1 protein purified from macrophages is active as a pro-inflammatory stimulus to macrophages, suggesting that, even without modification, HMGB1 protein can function as an inflammatory agent.

HMGB1 protein has been demonstrated to undergo acetylation on lysine 2 and 13 [31]. Acetylation of the N-terminal region of HMGB1 protein might modulate its functions, for example by altering the influence of the neighbouring C-terminal tail domain that has been previously shown to play a key role in HMGB1 protein function [32]. Trichostatin A (TSA), a general deacetylase inhibitor, produces a clear interference in transcriptional HMGB1 activity by suppressing the binding of HMGB1 to chromatin. We can surmise that if HMGB1 protein is maintained in an acetylated state it is deleterious to activated transcription.

Although HMGB1 protein was first classified as a nuclear protein associated with chromatin and most of the investigations on its function were concerned with its action in the nucleus, biochemical and immunological studies indicated that in certain cells, e.g. monocyte/macrophages, HMGB1 protein is also found in cytoplasm [33]. This cytoplasmic form HMGB1 is released into the extracellular milieu by activated monocytes/macrophages (Fig. 1). The mechanism of HMGB1 protein secretion is largely unknown, but some evidence suggests that HMGB1 protein may be released via cytoplasmic organelles, endolysosomes

[34]. Following activation with inflammatory stimuli, monocytes/macrophages may release large amounts of HMGB1 protein, but only after a lag or delay of 12–16 h. HMGB1 protein released by monocytes/macrophages within the first 16 h is derived from pre-formed cellular pool; after that time, cellular synthesis of this protein increases *de novo* [11]. It was shown [35] that in monocytes/macrophages, HMGB1 protein shuttles actively between the nucleus and cytoplasm. Monocytes/macrophages acetylate HMGB1 protein extensively upon activation with lipopolysaccharide. Moreover, forced hyperacetylation of HMGB1 protein in resting monocyte/macrophages causes its relocalization to the cytosol. Cytosolic HMGB1 is then concentrated by default into secretory lysosomes, and secreted when monocyte/macrophages receive an appropriate second signal. Such second signal may be IFN- γ . IFN- γ plays an important role in the regulation of HMGB1 protein release through a TNF- α and Janus kinase, 2-dependent mechanisms [36].

Released HMGB1 protein possesses a membrane-bound activity and mediates cellular proliferation and growth by signaling through the receptor for advanced glycation end products (RAGE). Interaction of HMGB1 protein with RAGE activates intracellular signal transduction through mechanisms involving nuclear factor κ B (NF κ B), mitogen-activated protein (MAP) kinases, plasminogen acti-

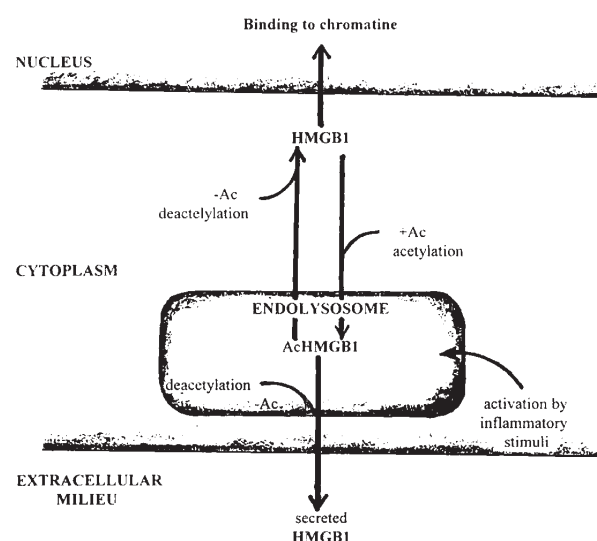


Fig. 1. Likely mechanism of HMGB1 protein secretion by activated macrophages. AcHMGB1 – acetylated form of HMGB1.

vation and proteins belonging to the Rho family (Cdc42, and Rac-1) [20]. HMGB1 protein increases the nuclear translocation of NF- κ B and enhances the expression of pro-inflammatory cytokines in human neutrophils. These pro-inflammatory effects of HMGB1 protein in neutrophils appear to involve the p38 MAPK, phosphatidylinositol 3-kinase/Akt, and ERK1/2 pathways. The mechanisms of HMGB1-induced neutrophil activation are distinct from endotoxin-induced signals, because HMGB1 protein leads to a different profile of gene expression, pattern of cytokine expression, and kinetics of p38 MAPK activation compared with LPS [37]. A recent study suggests that there may be additional cell surface receptor(s) (other than RAGE) with which HMGB1 interacts [38].

Serum HMGB1 levels are < 5ng/ml in the serum of healthy animals and normal human subjects [20]. Very high circulating levels of HMGB1 protein (up to 150 ng/ml) are observed in septic patients who died [11]. Hemorrhagic shock is also associated with significantly increased serum levels of HMGB1, even in the absence of infection or endotoxemia [39]. This finding indicates that the stimulus to HMGB1 protein release may be either pro-inflammatory cytokines released by ischemia or ischemia-induced cell injury.

Recent structure-function activity studies of HMGB1 reveal that the A and B boxes have unique properties [26]. The cytokine-stimulating domain of HMGB1 protein is localized in the B box, because recombinant, highly purified B box recapitulates the pro-inflammatory and macrophage-stimulating activities of full-length HMGB1 protein. In contrast, purified recombinant HMGB1 A box antagonizes the pro-inflammatory activity of full-length HMGB1 and of the B box itself. The A box can function as a competitive antagonist of HMGB1 protein binding to target cells (e.g., macrophages), and it remains possible that the A box may also interact with an as-yet-unidentified anti-inflammatory receptor. Little is known about whether endogenous biologic processing of full-length HMGB1 protein leads to the release of isolated A boxes and B boxes.

HMGB1 PROTEIN AS A PRO-INFLAMMATORY AGENT

It is now clear that HMGB1 protein occupies a unique position in the pro-inflammatory mediator cascade. HMGB1 has been termed a late mediator of endotoxin, lipopolysaccharide (LPS) lethality, because its release is delayed by several hours compared with other pro-inflammatory cytokines that mediate shock and tissue injury [11]. Direct evidence for the pro-inflammatory role of HMGB1 protein was obtained by administering highly purified, recombinant HMGB1 protein to normal animals and observing the development of inflammation and toxicity. HMGB1 is lethal to mice, and the toxicity of HMGB1 protein synergistically increases the lethality of endotoxins [11]. Anti-HMGB1 antibodies conferred significant protection against lethality even when the first dose of antibodies administered after the early TNF- α and IL1- β responses has resolved. Anti-HMGB1 protein antibodies have proved to be beneficial when therapy is initiated prior to the onset of increased HMGB1 levels, a treatment window of 12–6 h. Antibodies against HMGB1 protein conferred against the development of endotoxin-induced acute lung injury, indicating that HMGB1 is an endogenous mediator of endotoxin lethality and acute lung injury [12].

Fink [40] has recently identified ethyl pyruvate as an experimental therapeutic agent that protects animals against ischemia/reperfusion tissue injury and lethal hemorrhagic shock. Ethyl pyruvate is a stable lipophilic pyruvate derivative that is commonly used as a food additive and is regarded by the US Food and Drug Administration as generally regarded as safe (GRAS) substance. Reasoning that sepsis, ischemia/reperfusion, and hemorrhagic shock can each induce HMGB1 protein release, Tracey's group [41–43], investigated the effects of ethyl pyruvate on HMGB1 release and survival in cecal ligation and puncture (CLP)-induced sepsis. Ethyl pyruvate administration rescues animals from peritonitis-induced lethal sepsis, even when treatment is initiated as late as 24 h after cecal puncture surgery. Thus, *in vivo* studies, using either anti-HMGB1 antibodies or ethyl pyruvate suggest an important roles for

HMGB1 as a pro-inflammatory cytokine and as a therapeutic target in acute systemic inflammatory syndromes. Recent observation indicated that HMGB1 protein has pro-inflammatory activity *in vitro* [44]. Addition of highly purified recombinant HMGB1 to macrophage cultures activates the release of TNF- α , IL-1 α , IL-1 β , IL-1RA, IL-6, IL-8, macrophage inflammatory protein (MIP)-1 α , and MIP-1 β ; but not IL-10 and IL-12. HMGB1 protein concentrations that activated macrophages were within the pathological range previously observed in endotoxemic animals, and in serum obtained from septic patients [11]. HMGB1 protein failed to stimulate cytokine release in lymphocytes, indicating that cellular stimulation was specific. The kinetics of macrophage activation induced by HMGB1 are delayed relative to those of classic macrophage-activating factors (e.g., LPS or TNF- α), because addition of HMGB1 protein to macrophage cultures leads to increased TNF- α release after a significant delay. HMGB1-induced up-regulation of TNF- α mRNA and protein is biphasic, peaking 4 h and again 10 h after addition HMGB1 [22,44].

Ulloa et al. [45] have shown that ethyl pyruvate inhibited the release of TNF- α and HMGB1 protein from endotoxin stimulated murine macrophages and prevented the accumulation of serum HMGB1 levels in mice with lethal sepsis. Macrophages contain large quantities of HMGB1 that are released during activation through mechanisms that remain unknown. Ethyl pyruvate, which inhibits signaling pathways through both the p38 mitogen-activated protein kinase (MAPK) and NF- κ B, specifically prevented the release of HMGB1, because this compound did not alter intracellular steady state levels of HMGB1 protein. The protective effects occurred in therapeutically achievable, safe doses.

HMGB1 protein is a primary mediator of inflammation in the setting of cell injury or necrosis. It is widely known that products of injured or damaged cells (as occurs in trauma, cell death, or ischemic injury) activate TNF- α release from macrophages and other cells of the innate immune system, but the identity of stimulating factor(s) released from the injured cells has until now been enigmatic. Recent evidence using cells devoid of HMGB1 protein by

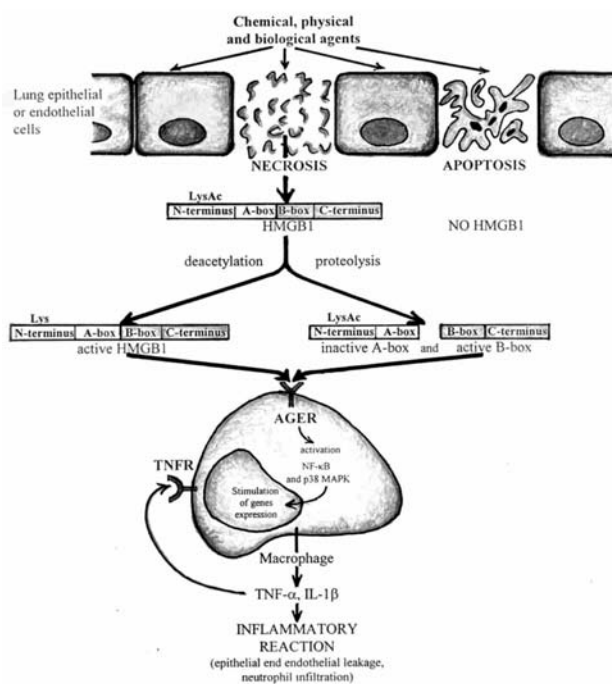
gene knockout reveals that deletion of HMGB1 renders cell lysates significantly less active in stimulating TNF- α secretion from monocyte/macrophages [46]. Moreover, cells activated to undergo apoptosis release significantly less HMGB1 protein compared with necrotic cells, and the diminished HMGB1 release corresponds to decreased TNF- α -inducing activity. Histone deacetylation in apoptotic cells appears to be associated with structural changes in the interaction between DNA and HMGB1 protein, resulting in decreased HMGB1 mobility and release [46].

When the data implicating HMGB1 protein as a systemic mediator of inflammation are considered, it appears that HMGB1 is a primary mediator of the acute inflammatory response to cell injury. It is plausible that HMGB1 protein may be an “integrator” of systemic inflammatory responses, because cell injury from a variety of sources may contribute to systemic increases in HMGB1 protein. For example, HMGB1 levels derived from activated monocytes, from ischemic or injured cells, or from necrosis, can cause a systemic inflammatory response in clinically diverse states, including endotoxemia, sepsis, and multiple organ injury from trauma or infection [47,48].

An important pathobiologic activity of HMGB1 protein was recently revealed in studies performed by Sappington et al. [19], which implicated HMGB1 as a mediator of epithelial barrier dysfunction. Exposure of epithelial cell monolayers to HMGB1 caused the monolayers to become “leaky”, so that large, normally excluded molecules passed through the cell layer. Epithelial leakage mediated by HMGB1 protein was not attributable to toxicity, but required signaling through MAP kinases, NF- κ B, and nitric oxide. This finding reveals a potentially important mechanism of HMGB1 toxicity. It is likely that epithelial dysfunction, occurring systemically in response to excessive HMGB1 levels, underlies the development of lethality when serum HMGB1 levels are elevated.

HMGB1 PROTEIN AND ACUTE LUNG INFLAMMATION

HMGB1 protein is likely to be one of the main diffusible signals of necrosis and acute inflammatory lung injury.



AGER – receptor for advanced glycation end products; NF- κ B – nuclear factor κ B; p38 MAPK – p38 mitogen-activated protein kinase; TNF- α – tumor necrosis factor- α ; IL-1 β – interleukin-1 β ; TNFR – TNF- α receptor.

Fig. 2. The current concepts of structure, function and activity of HMGB1 protein.

Figure 2 summarizes some of current concepts of structure, function, and activity of HMGB1 protein.

To determine whether HMGB1 might induce acute lung injury, Abraham et al. [12] used intratracheal administration of HMGB1 to mice. Lung tissue levels of IL-1 β , TNF- α , and macrophage-inflammatory protein (MIP)-2, were significantly elevated in a dose-dependent manner 8 h after HMGB1 administration, starting with doses as low as 1 μ g/mouse. Histological examination of tissue sections prepared from the lungs of mice treated with HMGB1 revealed an acute diffuse inflammatory response, with accumulation of neutrophils in the interstitial and intraalveolar space. These pathological changes are typically observed in response to acute lung injury mediated by endotoxin, TNF- α , IL-1, and other pro-inflammatory stimuli [49,50]. Treatment of mice with anti-HMGB1 antibodies before or after endotoxin exposure significantly attenuated the severity of lung edema produced by intratracheal instillation of endotoxin [12]. It was theoretically possible that the protective effects of anti-HMGB1 protein antibod-

ies were due to the decreased expression of TNF- α , IL-1 β and MIP-2 in the lung tissue. However, anti-HMGB1 treatment had no effect on endotoxin-induced increase in these cytokines. Thus, the protection by anti-HMGB1 in endotoxin-induced acute lung injury is specific.

HMGB1 protein has been identified as a late mediator of endotoxin lethality, because its systemic release during endotoxemia is delayed as compared with the rapid increase in the early pro-inflammatory cytokines, such as IL-1 β and TNF- α [11]. Delayed release of HMGB1 protein can also participate in the downstream development of lung injury [12]. This role of HMGB1 in pathogenesis of acute lung injury appears to be distinct from any effects on earlier acting pro-inflammatory cytokines. According to Wang et al. [51], pro-inflammatory cytokines, including IL-1 β and TNF- α , induce production of HMGB1. This is consistent with HMGB1 protein being a distal inflammatory mediator with delayed release after cellular exposure to endotoxin, or more likely, with release induced primarily by IL-1 β and TNF- α , the expression of which is rapidly increased by endotoxin. The delayed kinetics of HMGB1 release, associated with its contributory role in acute lung injury, point to HMGB1 protein as a potential target for therapeutic intervention. However, because inhibition of HMGB1 (e.g., anti-HMGB1 antibodies or ethyl pyruvate) does not completely prevent inflammatory injury to the lungs, it is likely that effective strategies for acute lung injury should focus on inhibiting the pathological effects of both early (e.g., TNF- α and IL-1 β) and late (e.g., HMGB1) mediators.

Lung endothelium cells appear to be the first cells of the lung to be altered in acute lung inflammation triggered by sepsis, trauma, and other systemic conditions [52,53]. The lung endothelium, in concert with the epithelial barrier, mediates the initial change in permeability and is also critical for repair and remodeling of the alveolar capillary membrane. Activation of endothelial cells, with functional changes involving both new gene expression and constitutive pathways that do not require new gene products, occurs in both pulmonary and systemic endothelia [1]. Such cellular processes as apoptosis and necrosis are likely to be central to imbalances between resolution and repair ver-

sus persistence and progression in acute lung inflammation and can be mainly influenced by inflammatory stimuli [54]. It is commonly believed that necrotic cells are pro-inflammatory. Certainly most pathological lesions characterized by necrosis are also characterized by inflammation. Phagocytosis of apoptotic cells by lung macrophages has been suggested to be a quiet process that does not lead to production of inflammatory mediators in lung tissues [9]. According to Scaffidi et al. [46] the passive release of an abundant chromatin component can serve as a diffusible signal of unprogrammed death (necrosis). Apoptotic cells neither result from present and immediate danger, nor trigger inflammation in physiological conditions. They retain nuclear components until cleared by lung macrophages or nearby cells that act as semi-professional phagocytes (e.g., lung epithelial cells), which they attract and activate by showing "eat me" signals. Core histones, although more abundant, would probably not be good signals of necrosis, as they remain anchored to the insoluble chromatin of necrotic cells. HMGB1 protein is both a nuclear factor and a secreted protein and is passively released from necrotic cells. HMGB1 protein is released from necrotic cells to the extracellular medium (promoting inflammation) because chromatin deacetylation is inhibited. In apoptotic cells, HMGB1 protein is bound firmly to chromatin. Hypoacetylation of chromatin or components may occur during apoptosis and favors HMGB1 binding in nucleus. This indicate that in apoptosis and necrosis chromatin undergoes some chemical or structural transition that makes it susceptible or non-susceptible to HMGB1 binding. The amount of HMGB1 protein bound to apoptotic and non-apoptotic chromatin was determined by immunoblotting using an antibody to HMGB1 protein [46].

HMGB1 protein binding to chromatin depends on the viability of the cell and clearly distinguishes necrotic from apoptotic cells. The intriguing interpretation of these results is that "spilled chromatin" and, more specifically, mobility of HMGB1 serves as a signal that determines whether cell death and injury lead to necrosis or continue along a preinitiated apoptotic pathway. The difference between cell death by necrosis and apoptosis is very significant. Necrosis is associated with organ deterioration,

whereas apoptosis is important for organ maintenance and differentiation. The status of nuclear HMGB1 protein determines which cell death pathway will develop. The differential release of HMGB1 protein by necrotic and apoptotic cells might be exploited as a cue to nearby cells to activate the appropriate responses to programmed (apoptosis) and unprogrammed cell death (necrosis) [11,12,44]. Necrosis is usually the result of trauma, poisoning or infection, each of which requires prompt reaction, damage containment and/or damage repair. To test directly whether the release of HMGB1 by necrotic cells might be an immediate trigger for an inflammatory response, Scaffidi et al. [46] challenged wild-type bone marrow cells with *Hmgb1*^{-/-} or wild-type dead fibroblasts (*Hmgb1*^{+/+}). Wild type necrotic cells triggered the production of the pro-inflammatory cytokine TNF- α by cultured macrophages, whereas wild-type apoptotic cells were much less effective. Significantly, *Hmgb1*^{-/-} necrotic cells were also ineffective in activating macrophages.

Massive release of diffusable HMGB1 from the nuclei of damaged or necrotic lung cells initiates a cascade of events that lead to cell death and lung deterioration. HMGB1 protein stimulates monocytes/macrophages to produce and release not only TNF- α and IL-1 β , but also to release HMGB1 [45]. Monocytes/macrophages contain large quantities of HMGB1 protein that are released during activation through a mechanism that remains unknown. It is possible that HMGB1 released from necrotic lung epithelial cells may activate monocytes/macrophages by binding to the receptor for advanced glycation end products (RAGE) [55]. HMGB1 protein binds to RAGE with sevenfold higher affinity compared to previously known RAGE ligands, advanced glycation end products [36]. Rendon-Mitchell et al. [44] considered that signaling through nuclear factor-kappaB (NF- κ B) and/or the p38 mitogen-activated protein kinase (MAPK) pathway may underline the regulation of HMGB1 release from activated macrophages. It is unclear how signaling through RAGE, which activates intracellular signal transduction pathways within minutes, can lead to a delayed expression of TNF- α mRNA occurring several hours later.



HMGB1 protein is constitutively expressed in quiescent macrophages to maintain a large preformed pool of HMGB1 in both the nucleus and cytoplasm regions. It has been observed that upon stimulation of macrophages, the intracellular levels of HMGB1 mRNA and protein remain unchanged, but HMGB1 protein is found predominantly in the cytoplasm as numerous aggregated granules. Likely activated monocytes/macrophages translocated HMGB1 from the nucleus to cytoplasmic organelles for subsequent active secretion [34].

Peak HMGB1 serum activity is detected 12–18 h after disease onset; in contrast, circulating TNF- α and IL-1 β levels typically revert to near-baseline levels within the first few hours of disease progression. These observations suggest that the activity of HMGB1 occurs downstream of TNF- α and other inflammatory mediators. It is now apparent that HMGB1 can participate in “cross-talk” for the propagation and amplification of downstream pro-inflammatory responses.

The release of HMGB1 protein by activated macrophages, its activity as a macrophage-stimulating agent and its causative role in lethal endotoxemia and sepsis, reveal that HMGB1 may be regarded as a late mediator of acute lung inflammation. We would like to suggest that HMGB1 may be also a mediator of the inflammatory response to lung cell injury caused by toxic environmental chemicals.

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