The role of high mobility group box-1 protein in severe sepsis
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**Purpose of review**

Despite medical advances, mortality in severe sepsis remains high. As our understanding of the innate immune system has expanded, clinical trials have focused on inhibiting cytokines present early in the infectious process such as interleukin-1 and tumor necrosis factor-alpha, although with disappointing results. There is evidence that the nuclear protein high mobility group box-1 protein, when released extracellularly, acts as a persistent mediator of sepsis and is therefore a promising candidate for therapeutic intervention. This review summarizes current knowledge of the protein and highlights recent relevant findings.

**Recent findings**

High mobility group box-1 protein may be released into the circulation either due to necrosis of cells or by active release from macrophages and endothelial cells. Models of experimental sepsis in mice have shown a strong association between extracellular high mobility group box-1 protein and lethality. Treatments against the biological activities of high mobility group box-1 protein reduce lethality in these models. Other studies have shown high mobility group box-1 protein as a key regulator in acute and chronic inflammation. Recent findings confirm that high mobility group box-1 protein is persistently elevated in human patients with severe sepsis.

**Summary**

Despite all efforts, mortality in severe sepsis remains high. A massive amount of evidence indicates high mobility group box-1 protein as a delayed and important propagator of inflammation. Recent studies confirm persisting high levels of high mobility group box-1 protein in serum up to 1 week after hospitalization. Reducing levels of the protein by anti-high mobility group box-1 protein treatment may be one way to moderate uncontrolled inflammation seen in sepsis.

**Keywords**

high mobility group box-1 protein, inflammation, sepsis, septic shock

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**Introduction**

In severe sepsis and septic shock the normally tightly controlled balance between the inflammatory, coagulatory, and neuroendocrine systems is lost. Despite antibiotics and modern intensive care, severe sepsis has a mortality rate of around 30%, resulting in an estimated 250,000 deaths annually in the United States [1]. Our understanding of the causes, mitigating factors, and mediators of severe sepsis has advanced in the last years, and a number of new therapies developed; intravenous immunoglobulin-G in streptococcal infections [2], low-dose corticosteroids [3] and activated protein C [4] all affect the inflammatory response seen during severe sepsis or septic shock. Many other anti-inflammatory interventions, some of them specifically directed against cytokines, appeared promising in animal studies, but did not translate well into human clinical trials [5]. Early strategies, for example, focused on blocking the actions of interleukin (IL)-1, [6] or tumor necrosis factor (TNF)-\alpha [7,8], but none of these early trials led to improved survival. There has been renewed interest in therapy directed against TNF-\alpha since a recent clinical trial reported an absolute mortality reduction of around 5% (in those treated with anti-TNF-\alpha antibodies vs. placebo) in a select group of patients with severe sepsis and elevated levels of IL-6 [9].

In severe sepsis, patients often succumb to death long after initial infection. In 1999, in an experiment designed to identify possible late mediators of sepsis, Wang et al. [10] found that macrophages stimulated with lipopolysaccharide (LPS) secreted a protein beginning at 16 h – long after the peaks of previously studied proinflammatory cytokines. The protein was identified as the nuclear protein high mobility group box-1 protein (HMGB1), and it was further demonstrated that administrating anti-HMGB1 antibodies, in an experimental model of murine endotoxemia, could reduce lethality from 100 to 30%. These promising findings dramatically increased the interest in HMGB1 as a proinflammatory cytokine.
High mobility group box-1 protein: the intranuclear protein

HMGB1 is a 215 amino acid protein, encoded on chromosome 13q12, which is highly conserved between species. There is 99% species homology between rodents and humans, the proteins differing by only two amino acids. It has three domains: two internal repeats of positively charged residues, the A-box and B-box, and a negatively charged terminal COOH terminus (Fig. 1). The two boxes bind to the minor groove of chromatin thus modifying the DNA architecture. This facilitates the binding of regulatory proteins, including various gene transcription factors, to form stable complexes with the DNA. It is likely to play a role in DNA repair and replication [11]. Interestingly, HMGB1 is not essential for fetal development as HMGB−/− mice are born full-term; however, they die shortly after birth due to hypoglycemia [12].

High mobility group box-1 protein: the extracellular protein

The extracellular functions of HMGB1 have long been recognized, particularly concerning its role in the differentiation of cells, in neurite outgrowth [13] and in the governing of cell motility [14–16]. As a cytokine, HMGB1 is released from endotoxin stimulated monocytes and, in itself, has proinflammatory properties, inducing other cytokines and chemokines, including TNF-α, IL-1α, IL-1β, IL-1RA, macrophage inflammatory protein (MIP)-1α, MIP-1β, IL-6 and IL-8 [17]. It activates endothelial cells, inducing the release of chemokines and cytokines and the upregulation of adhesion molecules [18,19], thereby increasing the adhesion of neutrophils and monocytes to stimulated endothelia. It induces epithelial-cell barrier leakage in the gut [20] through the downregulation of cell-surface proteins responsible for the adhesion between adjacent epithelial cells. HMGB1 also mediates migration of monocytes [21] and smooth muscle cells [22]. Interestingly, it stimulates dendritic cell maturation [23] which implicates a role in the switch from the innate to the adaptive immune system. The pro-inflammatory activity of HMGB1 mainly derives from the B-box while the A-box only has limited pro-inflammatory activity [24].

The receptor for advanced glycated end-products (RAGE) has been identified as a major receptor for HMGB1 [14]. RAGE antibodies, as well as soluble RAGE [25], which acts as a competitive inhibitor, reduce the proinflammatory activities of HMGB1. Furthermore, RAGE−/− mice show a significantly reduced inflammatory response in tissues compared with wild-type animals, when injected intraperitoneally with HMGB1 (Mullins et al., submitted manuscript). HMGB1 binding to RAGE leads to activation of the nuclear factor-κB (NF-κB) pathway and also activation of extracellular signal-regulated kinase and P38 [26]. HMGB1 also binds to Toll-like receptors (TLR)-2 and TLR-4, both of which leads to a MyD88-dependent activation of NF-κB [27,28**].

Release of high mobility group box-1 protein

Release of HMGB1 is stimulated by Toll signaling. Thus most bacterial antigens, for example LPS, can trigger HMGB1 release. Release is also triggered by endogenous proinflammatory signals such as the cytokines TNF-α, IL-1β [10] or interferon (IFN)-γ [29]. Cells that actively release HMGB1 include monocytes [10], tissue macrophages [30], endothelial cells [31], enterocytes [32*], pitiuicytes [33], mature myeloid dendritic cells (DCs) and activated natural killer (NK) cells [34]. The mechanisms by which HMGB1 is exported from the nucleus to the extracellular environment are only partly understood. HMGB1 lacks a classic leader sequence and, like the IL-1 family, it is released through an endolysosomal vesicle-mediated pathway [35]. When actively released after stimulation, HMGB1 is heavily acetylated, an acetylation that occurs in the nucleus, and that prevents re-entry once it has reached the cytosol [30]. Other sources of extracellular HMGB1 are cells undergoing necrosis. HMGB1 is only loosely bound to chromatin and is easily set free during the necrotic process [36], triggering further inflammation. In cells undergoing apoptosis, by contrast, binding to chromatin is much stronger, possibly due to a generalized underacetylation of histone. Programmed cell death therefore causes no local inflammation [36].

High mobility group box-1 protein in experimental sepsis

The kinetics of HMGB1 release and accumulation has been studied in murine models of endotoxemia and also in so-called cecal ligation and puncture (CLP), an experimental setup intended to mimic spontaneous peritonitis. In the study by Wang et al. [10], animals were injected intraperitoneally with a LD50 dose of endotoxin after which HMGB1 rose in the circulation, starting at 8 h, increased until 16 h, and thereafter remained stable until 36 h. In a CLP model, HMGB1 started to rise approximately 18 h after induction of peritonitis, and remained elevated for more than 72 h [37]. The kinetics of HMGB1 release in these animal models is delayed compared to other well-studied proinflammatory cytokines such as TNF-α, IL-1β and IL-6. More importantly, death of
animals parallels the accumulation of HMGB1 in sera or plasma.

Treatment
In the endotoxemia model, passive immunization with anti-HMGB1 antibodies significantly protected against lethal doses of LPS, even if treatment was delayed until 2 h after exposure [10]. The effect of anti-HMGB1 antibodies was dose-dependent and sustained after the peak of circulating TNF-α had passed. A similar effect was demonstrated in the CLP model [37] in which both anti-HMGB1 and A-box of HMGB1 effectively improved survival even after a delay of 24 h. The A-box segment of the protein has only weak proinflammatory activity and is a competitive inhibitor of the much more active B-box. Ethyl pyruvate, a non-toxic food additive and an experimental anti-inflammatory agent [38] that in vitro inhibits the release of HMGB1 from macrophages, also decreases lethality when given to mice in these animal models. Finally, it has recently been demonstrated that treatment with anti-interferon (IFN)-γ reduces mortality in a rat CLP model, an effect that the authors attribute to the reduced levels of HMGB1 achieved with treatment [39]. Thus, HMGB1 inhibiting treatment, be it HMGB1 antibodies, the A-box of HMGB1, ethyl pyruvate or anti-IFN-γ, all reduce sepsis lethality in mice or rats even if treatment is delayed.

Other ways to ameliorate the effects of HMGB1 may be to develop treatments that focus on its receptors. One interesting observation, for example, is that RAGE−/− mice exhibit reduced inflammatory responses to injected HMGB1 and reduced lethality in CLP models compared to wild type mice [40].

High mobility group box-1 protein in organ disorders and diseases other than sepsis
Elevated levels of HMGB1 are seen in several organ disorders and diseases characterized by inflammatory responses (Table 1).

High mobility group box-1 protein in human sepsis
Three papers address the issue of HMGB1 in sepsis. The first was the seminal work by Wang et al. [33] that 7 years ago started the recent wave of research on HMGB1 as a late mediator of inflammation. Increased levels of HMGB1 were found in 25 critically ill patients with sepsis, and significantly higher levels in those that succumbed to disease compared to those that survived. The kinetics of HMGB1 release was not discussed in that paper. In 2005 another study [52*] took a closer look at the kinetics of HMGB1 and four other cytokines. Sixty-four patients with sepsis, severe sepsis or septic shock (for definitions, see Bone et al. [53]), of whom 10 succumbed, were studied. Blood samples were taken at five time points during a 144 h study period and HMGB1 was analyzed, along with severity of disease and a number of clinical and laboratory parameters, including IL-6, IL-8, IL-10 and TNF-α. As expected, HMGB1 remained persistently elevated in the majority of patients throughout the study period with serum concentrations at the final sampling point 300 times higher than any of the other studied cytokines (Fig. 2). This was consistent with theory and the previous publication. Contrary to expectations, and at odds with the Wang et al. paper, however, this study found no correlation between levels of HMGB1 and severity of disease as measured by acute physiology and chronic health evaluation (APACHE) II and sepsis-related organ failure assessment (SOFA)-scores. In fact, there was a tendency to higher levels of HMGB1 in those with severe sepsis compared to those with septic shock, and also significantly lower levels in those who perished within 28 days compared to those who survived, at least with one of the Western blot methods used. An important point to stress is that in all studies healthy controls have had low (<2 ng/ml) or non-detectable levels of circulating HMGB1. A third recently published study reports levels of circulating HMGB1 in 201 patients with suspected (40 confirmed) disseminated intravascular coagulation (DIC) [51*]. Forty, of whom eight perished, had a diagnosis of infection. It is difficult to compare this study with the other two for several reasons, the most important being the different case definitions. Patients with infections in the DIC study belong to a subgroup which is not easily broken out of the whole. The DIC study furthermore does not specify when during hospitalization blood samples were taken, nor are kinetics reported. The results and conclusions differ in some important regards from the other reports. In the group with infections in the DIC study, mean levels of HMGB1 are much lower than in the two other studies discussed (4.54 ± 8.18 ng/ml [mean ± SD] compared to 42.3 ± 82.8 ng/ml on admittance [52*] and 25.2 ng/ml in survivors, and 83.7 ng/ml in non-survivors [10], respectively). Whereas Sundén-Cullberg et al. [52*] looked particularly at severe sepsis and septic shock, and found no correlation between levels of HMGB1 and severity of disease, the DIC study does report such a correlation for its study population (suspected or verified DIC), and also higher levels in those who perished compared to survivors. Whether this observation holds true for infected patients is not reported.

One possible explanation for the diverging results is that in Wang et al. [10] and Sundén-Cullberg et al. [52*], Western blot was used to analyze sera, whereas in Hatada et al. [51*], an ELISA technique was utilized. This technique was first published by the same group in 2003 [54], but we have not found its use published by groups outside Japan. It should also be noted that
different Western blot techniques can yield different results [52]. It is possible that the different methods detect different subsets of the protein. In this discussion a further caveat is warranted; none of the three studies mentioned addresses the issue of where the HMGB1 originates – whether it is actively secreted from stimulated cells or passively released from necrotic ones – and, related to this, whether or not it is in acetylated form and if the protein measured is actually biologically active. The different results in these studies reflect some uncertainty concerning what are true levels of biologically active extracellular HMGB1 in septic patients, but we can safely state that levels are highly elevated in a majority of septic patients, long after they are admitted to the hospital. In the study that most carefully examined patients with severe sepsis and septic shock, however, there was no correlation to severity of disease.

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### Table 1: High mobility group box-1 protein in organ disorders and diseases other than sepsis

<table>
<thead>
<tr>
<th>Organ/disease</th>
<th>Occurrence of high mobility group box-1 protein</th>
<th>Effect of high mobility group box-1 protein</th>
<th>Effect of anti-high mobility group box-1 protein antibodies or other treatment</th>
</tr>
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<tbody>
<tr>
<td>Lung</td>
<td>Acute liver damage, following on interrupted blood perfusion or exposure to toxins, results in release of high mobility group box-1 protein [42]</td>
<td>Intra-tracheal administration of high mobility group box-1 protein in mice induces acute inflammation in the lung [41]</td>
<td>Anti-high mobility group box-1 protein treatment in experimental rheumatoid arthritis limits disease severity and progression [47]</td>
</tr>
<tr>
<td>Liver</td>
<td>High mobility group box-1 protein, along with other RAGE ligands, will limit liver regeneration [43]</td>
<td>High mobility group box-1 protein has proinflammatory activity within the central nervous system, inducing fever and lowered pain thresholds (alodynia) [44], aphagia and taste aversion [45]</td>
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<td>Rheumatoid arthritis</td>
<td>The presence of cytoplasmatic and extracellular high mobility group box-1 protein has been reported in both experimental arthritis models and in human rheumatoid arthritis [46]</td>
<td>Anti-high mobility group box-1 protein treatment in experimental rheumatoid arthritis limits disease severity and progression [47]</td>
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<td>Malaria</td>
<td>Patients with cerebral malaria, a disease which is characterized by an intense inflammatory response, have high circulating levels of high mobility group box-1 protein [48]</td>
<td>Anti-high mobility group box-1 protein treatment in experimental rheumatoid arthritis limits disease severity and progression [47]</td>
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<td>Chronic myositis</td>
<td>In muscle biopsies taken from patients with chronic myositis, high mobility group box-1 protein was detected cytoplasmatically and also extracellularly, surrounding the inflammatory infiltrates [49]</td>
<td>Anti-high mobility group box-1 protein treatment in experimental rheumatoid arthritis limits disease severity and progression [47]</td>
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Figure 2: The kinetics of high mobility group box-1 protein and four other cytokines during the first 144 h after admittance to hospital in 64 patients with sepsis, severe sepsis or septic shock.
There is one recently proposed idea that perhaps can help explain the divergent HMGB1 results. Two reports advance the theory that HMGB1 causes severe sepsis but not septic shock [55**, 56]. They also make the novel assertion that severe sepsis and septic shock are clinically and immunologically distinct syndromes. According to this view TNF-α mediates septic shock but not severe sepsis, in contrast to HMGB1 which supposedly causes severe sepsis but not septic shock. Evidence is supplied mainly from murine experiments. Mice that succumb to HMGB1 poisoning maintain normal blood pressure and heart rates until suddenly dying from cardiac standstill, whereas those exposed to lethal doses of TNF will develop hypotension, resulting in widespread organ dysfunction, ultimately leading to death. Necropsies of animals that have died from HMGB1 are reported to show very discrete pathological signs. In contrast, TNF-poisoned animals show widespread necrosis and inflammatory changes in internal organs.

Although persuasive, there remains a lot of clinical research before general acceptance in the medical community of the idea that severe sepsis and septic shock are pathophysiologically distinct syndromes in patients. In clinical practice, they are perceived and treated as closely intertwined entities.

Conclusion

Immunological interventions in the treatment of critical illness due to infections remain a promising but very complex subject. It is likely that subgroups of these patients would benefit from individually tailored immunomodulatory drugs. Considering the combined massive evidence from in-vitro work, animal studies, and the high levels of HMGB1 found in critically ill patients with infections and DIC, HMGB1-lowering or modifying substances are well placed to qualify as part of the therapeutic arsenal. However, given the lack of a clear correlation between HMGB1 and severity of disease in infections, further studies are needed to determine which septic patients might actually benefit from such therapy.

Acknowledgement

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest • • of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 298–299).


An excellent study that further describes HMGB1 signaling.


Further evidence of the involvement of HMGB1 in intestinal inflammation driven intestinal injury.


An interesting study that again shows the association of HMGB1 with critical illness.


A study describing the kinetics of HMGB1 in severe sepsis and septic shock.


An excellent, comprehensive and up to date review of HMGB1.