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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Abbreviations

CLP	cecal ligation and puncture
DIC	disseminated intravascular coagulation
HMGB1	high mobility group box-1 protein
RAGE	receptor for advanced glycated end-products

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)-α [7,8], but none of these early trials led to improved survival. There has been renewed interest in therapy directed against TNF-α since a recent clinical trial reported an absolute mortality reduction of around 5% (in those treated with anti-TNF-α 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.

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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•], pituicytes [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

Figure 1 Schematic representation of the structure of high mobility group box-1 protein
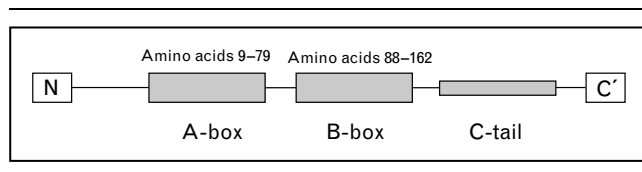
animals parallels the accumulation of HMGB1 in sera or plasma.

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 $\pm$ 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 Sunden-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 Sunden-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

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

Organ/disease	Occurrence of high mobility group box-1 protein	Effect of high mobility group box-1 protein	Effect of anti-high mobility group box-1 protein antibodies or other treatment
Lung		Intra-tracheal administration of high mobility group box-1 protein in mice induces acute inflammation in the lung [41]	
Liver	Acute liver damage, following on interrupted blood perfusion or exposure to toxins, results in release of high mobility group box-1 protein [42]	High mobility group box-1 protein, along with other RAGE ligands, will limit liver regeneration [43]	
Central nervous system	In human patients with purulent meningitis, high levels of high mobility group box-1 protein are seen in cerebrospinal fluid (Dumpis *et al.*, submitted manuscript)	High mobility group box-1 protein has proinflammatory activity within the central nervous system, inducing fever and lowered pain thresholds (allodynia) [44], aphagia and taste aversion [45]	
Rheumatoid arthritis	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]		Anti-high mobility group box-1 protein treatment in experimental rheumatoid arthritis limits disease severity and progression [47]
Malaria	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]		
Chronic myositis	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]		Systemic corticosteroid treatment led to diminished expression of high mobility group box-1 protein in this group [49]
Cancer	Upregulated in many types of tumor [50]		
Disseminated intravascular coagulation	Significantly elevated in the circulation of patients with verified disseminated intravascular coagulation [51•]		

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

■, high mobility group box-1 protein; ○, interleukin-6; □, interleukin-8; ◆, interleukin-10; ●, tumor necrosis factor-α.
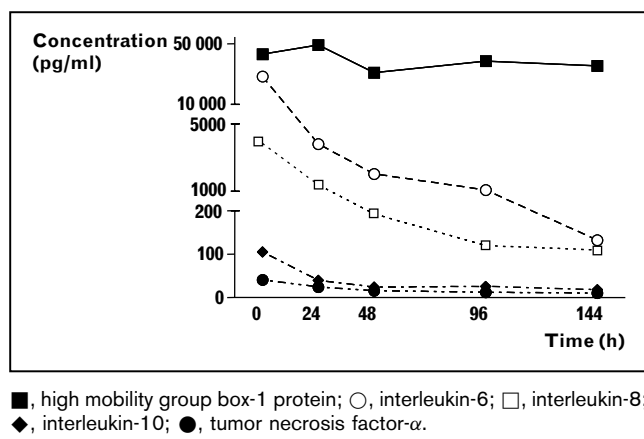
different Western blot techniques can yield different results [52•]. It is possible that the different methods detect different subsets of the protein. In this discussion a further caveat is warranted; none of the three studies mentioned addresses the issue of where the HMGB1 originates – whether it 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.

There is one recently proposed idea that perhaps can help explain the divergent HMGB1 results. Two reports advance the theory that 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

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).

1 Angus DC, Linde-Zwirble WT, Lidicker J, *et al.* Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001; 29:1303–1310.

2 Darenberg J, Ihendyane N, Sjolin J, *et al.* Intravenous immunoglobulin G therapy in streptococcal toxic shock syndrome: a European randomized, double-blind, placebo-controlled trial. Clin Infect Dis 2003; 37:333–340.

3 Annane D, Sebille V, Charpentier C, *et al.* Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA 2002; 288:862–871.

4 Bernard GR, Vincent JL, Laterre PF, *et al.* Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001; 344:699–709.

5 Riedemann NC, Guo RF, Ward PA. The enigma of sepsis. J Clin Invest 2003; 112:460–467.

6 Fisher CJ, Slotman GJ, Opal SM, *et al.* Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial. The IL-1RA Sepsis Syndrome Study Group. Crit Care Med 1994; 22:12–21.

7 Fisher CJ Jr, Agosti JM, Opal SM, *et al.* Treatment of septic shock with the tumor necrosis factor receptor: Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group. N Engl J Med 1996; 334:1697–1702.

8 Abraham E, Wunderink R, Silverman H, *et al.* Efficacy and safety of monoclonal antibody to human tumor necrosis factor alpha in patients with sepsis syndrome. A randomized, controlled, double-blind, multicenter clinical trial. TNF-alpha MAb Sepsis Study Group. JAMA 1995; 273:934–941.

9 Panacek EA, Marshall JC, Albertson TE, *et al.* Efficacy and safety of the monoclonal anti-tumor necrosis factor antibody F(ab′)2 fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels. Crit Care Med 2004; 32:2173–2182.

10 Wang H, Bloom O, Zhang M, *et al.* HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999; 285:248–251.

11 Bustin M. Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Mol Cell Biol 1999; 19: 5237–5246.

12 Calogero S, Grassi F, Aguzzi A, *et al.* The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. Nat Genet 1999; 22:276–280.

13 Rauvala H, Pihlaskari R. Isolation and some characteristics of an adhesive factor of brain that enhances neurite outgrowth in central neurons. J Biol Chem 1987; 262:16625–16635.

14 Merenmies J, Pihlaskari R, Laitinen J, *et al.* 30-kDa heparin-binding protein of brain (amphoterin) involved in neurite outgrowth. Amino acid sequence and localization in the filopodia of the advancing plasma membrane. J Biol Chem 1991; 266:16722–16729.

15 Rauvala H, Merenmies J, Pihlaskari R, *et al.* The adhesive and neurite-promoting molecule p30: analysis of the amino-terminal sequence and production of antipeptide antibodies that detect p30 at the surface of neuroblastoma cells and of brain neurons. J Cell Biol 1988; 107:2293–2305.

16 Parkkinen J, Raulo E, Merenmies J, *et al.* Amphoterin, the 30-kDa protein in a family of HMG1-type polypeptides. Enhanced expression in transformed cells, leading edge localization, and interactions with plasminogen activation. J Biol Chem 1993; 268:19726–19738.

17 Andersson U, Wang H, Palmblad K, *et al.* High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. J Exp Med 2000; 192:565–570.

18 Fiuza C, Bustin M, Talwar S, *et al.* Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. Blood 2003; 101: 2652–2660.

19 Treutiger CJ, Mullins GE, Johansson AS, *et al.* High mobility group 1 B-box mediates activation of human endothelium. J Intern Med 2003; 254:375–385.

20 Sappington PL, Yang R, Yang H, *et al.* HMGB1 B box increases the permeability of Caco-2 enterocytic monolayers and impairs intestinal barrier function in mice. Gastroenterology 2002; 123:790–802.

21 Rouhiainen A, Kuja-Panula J, Wilkman E, *et al.* Regulation of monocyte migration by amphoterin (HMGB1). Blood 2004; 104:1174–1182.

22 Degryse B, Bonaldi T, Scaffidi P, *et al.* The high mobility group (HMG) boxes of the nuclear protein HMG1 induce chemotaxis and cytoskeleton reorganization in rat smooth muscle cells. J Cell Biol 2001; 152:1197–1206.

23 Messmer D, Yang H, Telusma G, *et al.* High mobility group box protein 1: an endogenous signal for dendritic cell maturation and Th1 polarization. J Immunol 2004; 173:307–313.

24 Li J, Kokkola R, Tabibzadeh S, *et al.* Structural basis for the proinflammatory cytokine activity of high mobility group box 1. Mol Med 2003; 9:37–45.

25 Huttunen HJ, Fages C, Kuja-Panula J, *et al.* Receptor for advanced glycation end products-binding COOH-terminal motif of amphoterin inhibits invasive migration and metastasis. Cancer Res 2002; 62:4805–4811.

26 Kokkola R, Andersson A, Mullins G, *et al.* RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. Scand J Immunol 2005; 61:1–9.

27 Park JS, Svetkauskaite D, He Q, *et al.* Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem 2004; 279:7370–7377.

28 Park JS, Gamboni-Robertson F, He Q, *et al.* High Mobility Group Box 1 protein
•• (HMGB1) interacts with multiple Toll like receptors. Am J Physiol Cell Physiol 2006; 290:C917–C924. [Epub ahead of print 2 Nov 2005].
An excellent study that further describes HMGB1 signaling.

29 Rendon-Mitchell B, Ochani M, Li J, *et al.* IFN-gamma induces high mobility group box 1 protein release partly through a TNF-dependent mechanism. J Immunol 2003; 170:3890–3897.

30 Bonaldi T, Talamo F, Scaffidi P, *et al.* Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. EMBO J 2003; 22:5551–5560.

31 Mullins GE, Sunden-Cullberg J, Johansson AS, *et al.* Activation of human umbilical vein endothelial cells leads to relocation and release of high-mobility group box chromosomal protein 1. Scand J Immunol 2004; 60:566–573.

32 Liu S, Stolz DB, Sappington PL, *et al.* HMGB1 is secreted by immunosti-
• mulated enterocytes and contributes to cytomix-induced hyperpermeability of caco-2 monolayers. Am J Physiol Cell Physiol 2005. [Epub ahead of print 9 Nov 2005].
Further evidence of the involvement of HMGB1 in intestinal inflammation driven intestinal injury.

33 Wang H, Vishnubhakat JM, Bloom O, *et al.* Proinflammatory cytokines (tumor necrosis factor and interleukin 1) stimulate release of high mobility group protein-1 by pituicytes. Surgery 1999; 126:389–392.

34 Semino C, Angelini G, Poggi A, Rubartelli A. NK/iDC interaction results in IL-18 secretion by DCs at the synaptic cleft followed by NK cell activation and release of the DC maturation factor HMGB1. Blood 2005; 106:609–616.

35 Gardella S, Andrei C, Ferrera D, *et al.* The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. EMBO Rep 2002; 3:995–1001.

36 Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002; 418:191–195.

37 Yang H, Ochani M, Li J, *et al.* Reversing established sepsis with antagonists of endogenous high-mobility group box 1. Proc Natl Acad Sci U S A 2004; 101:296–301.

38 Ulloa L, Ochani M, Yang H, *et al.* Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. Proc Natl Acad Sci U S A 2002; 99:12351–12356.

39 Yin K, Gribbin E, Wang H. Interferon-gamma inhibition attenuates lethality after cecal ligation and puncture in rats: implication of high mobility group box-1. Shock 2005; 24:396–401.

40 Liliensiek B, Weigand MA, Bierhaus A, *et al.* Receptor for advanced glycation end products (RAGE) regulates sepsis but not the adaptive immune response. J Clin Invest 2004; 113:1641–1650.

41 Abraham E, Arcaroli J, Carmody A, *et al.* HMG-1 as a mediator of acute lung inflammation. J Immunol 2000; 165:2950–2954.

42 Tsung A, Sahai R, Tanaka H, *et al.* The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med 2005; 201:1135–1143.

43 Cataldegirmen G, Zeng S, Feirt N, *et al.* RAGE limits regeneration after massive liver injury by coordinated suppression of TNF-alpha and NF-kappaB. J Exp Med 2005; 201:473–484.

44 O'Connor KA, Hansen MK, Rachal Pugh C, *et al.* Further characterization of high mobility group box 1 (HMGB1) as a proinflammatory cytokine: central nervous system effects. Cytokine 2003; 24:254–265.

45 Agnello D, Wang H, Yang H, *et al.* HMGB-1, a DNA-binding protein with cytokine activity, induces brain TNF and IL-6 production, and mediates anorexia and taste aversion. Cytokine 2002; 18:231–236.

46 Kokkola R, Sundberg E, Ulfgren AK, *et al.* High mobility group box chromosomal protein 1: a novel proinflammatory mediator in synovitis. Arthritis Rheum 2002; 46:2598–2603.

47 Kokkola R, Li J, Sundberg E, *et al.* Successful treatment of collagen-induced arthritis in mice and rats by targeting extracellular high mobility group box chromosomal protein 1 activity. Arthritis Rheum 2003; 48:2052–2058.

48 Alleva LM, Yang H, Tracey KJ, Clark IA. High mobility group box 1 (HMGB1) protein: possible amplification signal in the pathogenesis of falciparum malaria. Trans R Soc Trop Med Hyg 2005; 99:171–174.

49 Ulfgren AK, Grundtman C, Borg K, *et al.* Down-regulation of the aberrant expression of the inflammation mediator high mobility group box chromosomal protein 1 in muscle tissue of patients with polymyositis and dermatomyositis treated with corticosteroids. Arthritis Rheum 2004; 50:1586–1594.

50 Lotze MT, DeMarco RA. Dealing with death: HMGB1 as a novel target for cancer therapy. Curr Opin Investig Drugs 2003; 4:1405–1409.

51 Hatada T, Wada H, Nobori T, *et al.* Plasma concentrations and importance of
• High Mobility Group Box protein in the prognosis of organ failure in patients with disseminated intravascular coagulation. Thromb Haemost 2005; 94:975–979.
An interesting study that again shows the association of HMGB1 with critical illness.

52 Sunden-Cullberg J, Norrby-Teglund A, Rouhiainen A, *et al.* Persistent eleva-
• tion of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. Crit Care Med 2005; 33:564–573.
A study describing the kinetics of HMGB1 in severe sepsis and septic shock.

53 Bone RC, Balk RA, Cerra FB, *et al.* Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/ Society of Critical Care Medicine. Chest 1992; 101:1644–1655.

54 Yamada S, Inoue K, Yakabe K, *et al.* High mobility group protein 1 (HMGB1) quantified by ELISA with a monoclonal antibody that does not cross-react with HMGB2. Clin Chem 2003; 49:1535–1537.

55 Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear
•• weapon in the immune arsenal. Nat Rev Immunol 2005; 5:331–342.
An excellent, comprehensive and up to date review of HMGB1.

56 Czura CJ, Yang H, Amella CA, Tracey KJ. HMGB1 in the immunology of sepsis (not septic shock) and arthritis. Adv Immunol 2004; 84:181–200.