Published in final edited form as:
Viral Immunol. 2006 ; 19(1): 3–9.

Potential Role of High Mobility Group Box 1 in Viral Infectious Diseases

HAICHAO WANG¹, MARY F. WARD¹, XUE-GONG FAN², ANDREW E. SAMA¹, and WEI LI¹

¹Department of Emergency Medicine, North Shore University Hospital–New York University School of Medicine, Manhasset, New York.

²Department of Infectious Diseases, Xiangya Hospital, Central South University, Changsha, China.

Abstract

A nuclear protein, high mobility group box 1 (HMGB1), is released passively by necrotic cells and actively by macrophages/monocytes in response to exogenous and endogenous inflammatory stimuli. After binding to the receptor for advanced glycation end products (RAGE), or Toll-like receptor 4 (TLR4), HMGB1 activates macrophages/monocytes to express proinflammatory cytokines, chemokines, and adhesion molecules. Pharmacological suppression of its activities or release is protective against lethal endotoxemia and sepsis, establishing HMGB1 as a critical mediator of lethal systemic inflammation. In light of observations that many viruses (e.g., West Nile virus, Salmon anemia virus) can induce passive HMGB1 release, we propose a potential pathogenic role of HMGB1 in viral infectious diseases.

INTRODUCTION

Anon-histone nucleosomal protein was purified from nuclei approximately 30 years ago and termed “high mobility group 1” (HMG-1), or high mobility group box 1 (HMGB1), based on its rapid mobility on electrophoresis gels (58). It is constitutively expressed in quiescent cells, and a large “pool” of preformed HMGB1 is stored in the nucleus (9), because of the presence of two lysine-rich nuclear localization sequences (5). As an evolutionarily conserved protein, HMGB1 shares 100% homology (in amino acid sequence) between mouse and rat, and a 99% homology between rodent and human being (58).

HMGB1 contains two internal repeats of positively charged domains (“HMG boxes,” known as “A box” and “B box”) in the N-terminus, and a continuous stretch of negatively charged (aspartic and glutamic acid) residues in the C-terminus (Fig. 1). It is capable of binding chromosomal DNA, and has been implicated in diverse cellular functions, including determination of nucleosomal structure and stability, and binding of transcription factors to their cognate DNA sequences (34). In addition to the nucleus, HMGB1 is also localized to the cell membrane of neuronal (neuroblastoma) cells, where it co-localizes and interacts with the receptor for advanced glycation endproducts (RAGE) (22).

Recently we discovered that HMGB1 is released by activated macrophages/monocytes, and functions as a late mediator of lethal endotoxemia and sepsis (52,55,58,61). The recent discovery of HMGB1 as a critical mediator of inflammation diseases has stimulated tremendous interest in the field of inflammation research. In this article, we provide an

Correspondence to: HAICHAO WANG.

Address reprint requests to: Dr. Haichao Wang Department of Emergency Medicine, North Shore University Hospital–New York University School of Medicine 350 Community Drive Manhasset, NY 11030 E-mail: hwang@nshs.edu.

overview of recent advances in uncovering its extracellular role as a proinflammatory cytokine, and discuss its potential roles in viral infection-elicited inflammatory responses.

RELEASE OF HMGB1

Active secretion

Quiescent macrophages/monocytes constitutively express HMGB1, and maintain an intracellular “pool” of HMGB1 predominantly in the nucleus (Fig. 2) (9). After stimulation with exogenous bacterial products such as endotoxin, or with endogenous proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1 β , and interferon (IFN)- γ , cultures of macrophages, monocytes, and pituocytes actively release HMGB1 in a time- and dose-dependent manner (8,42,52,56). Lacking a signal sequence in the N-terminus, HMGB1 cannot be released via the classical endoplasmic reticulum-Golgi secretory pathway. Instead, activated macrophages/monocytes acetylate HMGB1 at potential nuclear localization sequences, leading to its cytoplasmic translocation and subsequent release into the extracellular milieu (Fig. 2) (5,8,9,20,42).

The kinetics of HMGB1 accumulation in vivo has been studied in murine models of endotoxemia and sepsis (induced by cecal ligation and puncture, CLP). Serum HMGB1 was first detectable 8 h after the onset of lethal endotoxemia and experimental sepsis, increased to plateau levels from 16 to 32 h, and remained elevated for at least 72 h (52,61). The late appearance of HMGB1 parallels with the onset of animal lethality from endotoxemia or sepsis, and distinguishes itself from TNF and other early proinflammatory cytokines (57). In septic patients, serum HMGB1 levels have been found to be elevated (46,52), and to be significantly higher in septic patients who did not survive as compared to survivors (52).

Passive leakage

In addition to its active release from innate immune cells, HMGB1 can also be released passively from necrotic or damaged cells (16,44). HMGB1 released by necrotic cells is capable of inducing an inflammatory response, thereby transmitting the “injury” signal to neighboring immune cells (44). However, HMGB1 is not released by apoptotic cells (44), which disintegrate themselves without setting off an inflammatory response. For instance, HMGB1 is released quickly after tissue ischemia/reperfusion injury (48), thereby functioning as an inflammatory mediator of tissue injury.

In an animal model of hemorrhagic shock, tissue HMGB1 levels are also significantly increased (26). Similarly, serum HMGB1 levels increased significantly in a patient with hemorrhagic shock, and returned toward basal levels as the clinical condition improved (36). The mechanisms of HMGB1 release in the absence of infection remain elusive, but are possibly attributable to active release from immune cells as well as passive leakage from damaged and dying cells (57). Therefore, HMGB1 might be a critical molecule that allows innate immune cells to respond to both infection and injury, thereby triggering a rigorous inflammatory response.

HMGB1 AS A PROINFLAMMATORY CYTOKINE

HMGB1 receptors

HMGB1 binds to RAGE (22), a member of the immunoglobulin superfamily of cell surface molecules expressed on mononuclear phagocytes, vascular smooth muscle cells, and neurons (22). Engagement of RAGE with ligands (such as AGEs or HMGB1) activates mitogen-activated protein kinase (MAPK) and NF- κ B (10,22), and induces production of various proinflammatory cytokines. The important role of RAGE in HMGB1-induced cytokine

production was supported by two observations. First, RAGE-neutralizing antibodies significantly attenuated HMGB1-induced TNF release (by 40–50%; H. Wang, unpublished). Second, depletion of RAGE expression partially attenuated HMGB1-induced production of proinflammatory cytokines (27).

Recent structural/functional studies have localized the cytokine functional motif (amino acids 106–123) in the “B box” but the RAGE-binding motif (amino acids 150–183) in the C-terminus (21,28,33), suggesting a potential involvement of other receptors for HMGB1-mediated macrophage activation (Fig. 1). Indeed, accumulating evidence has suggested a role for Toll-like receptor 4 (TLR4) as an alternative cell surface receptor for HMGB1 to activate innate immune cells (39). Consistently, Toll-like receptor 4 (TLR4)-defective (C3H/HeJ) mice are more resistant to HMGB1-mediated ischemic injury (48), supporting an important role for TLR4 in HMGB1-mediated inflammatory responses.

In vitro cytokine activities

HMGB1 stimulates macrophages, monocytes, and neutrophils to release proinflammatory cytokines (e.g., TNF, IL-1, IL-6, IL-8, and MIP-1) in a p38- and JNK MAPK-dependent mechanism (3,28,38). Similarly, in response to HMGB1 stimulation, human microvascular endothelial cells increase the expression of intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), proinflammatory cytokines (e.g., TNF), and chemokines (e.g., IL-8) (19,47), suggesting that HMGB1 can propagate an inflammatory response in the endothelium during infection or injury.

In vivo cytokine activities

Intratracheal administration of HMGB1 induces lung neutrophil infiltration, local production of proinflammatory cytokines (e.g., IL-1, and TNF), and acute lung injury (1,31,49). Intracerebroventricular application of HMGB1 induces brain TNF and IL-6 production, and sickness behaviors such as anorexia and taste aversion (2). Focal administration of HMGB1 near sciatic nerve induces unilateral and bilateral low threshold mechanical allodynia (6). Finally, intraperitoneal injection of HMGB1 increases ileal mucosal permeability, leading to bacterial translocation to mesenteric lymph nodes (43). Considered together, these studies indicate that accumulation of HMGB1 can amplify the cytokine cascade, and mediate injurious inflammatory responses.

Although excessive HMGB1 may be pathogenic, low levels of HMGB1 might still be beneficial. For instance, HMGB1 is capable of attracting stem cells (37), and may be needed for tissue repair and regeneration. A recent study demonstrated that HMGB1 facilitates myocardial cell regeneration after cardiac infarction, and consequently improves myocardial function (30). Therefore, like other proinflammatory cytokines, there may be protective advantages of extracellular HMGB1 when released at low amounts (29).

HMGB1 AS A THERAPEUTIC TARGET FOR EXPERIMENTAL SEPSIS

The important role of HMGB1 as a late mediator of lethal endotoxemia and sepsis (induced by CLP) has been established using HMGB1-specific neutralizing antibodies. Anti-HMGB1 antibodies dose-dependently protect mice against lethal endotoxemia (52), as well as endotoxin-induced acute lung injury (1,49). Furthermore, anti-HMGB1 antibodies rescue mice from lethal sepsis even when the first dose of antibodies is given as late as 24 h after CLP surgery (61).

An increasing number of agents (ethyl pyruvate, stearyl lysophosphatidylcholine, nicotine) dose-dependently inhibits HMGB1 release, and confers significant protection against lethal sepsis (9,15,50,53,55,60). Neutralizing antibodies against IFN- γ a cytokine capable of

stimulating HMGB1 release (42), significantly reduced circulating HMGB1 levels in septic rats and consequently rescued rats from lethal sepsis (62). Most recently, we discovered that green tea and aqueous extract of a Chinese herb, *Angelica sinensis*, significantly attenuated endotoxin-induced HMGB1 release (11,54) and rescued mice from lethal sepsis even when the first doses were given 24 h after the onset of sepsis (54). Notably, these anti-HMGB1 agents are capable of rescuing animals from lethal sepsis even when the first doses are given 24 h after the onset of sepsis (50,55,61), indicating a wider window for HMGB1-targeted therapeutic strategies.

POTENTIAL ROLES OF HMGB1 IN VIRAL INFECTIOUS DISEASES

Many viruses are not directly cytopathic but can trigger inflammatory responses as manifested by the production of various proinflammatory cytokines. When produced at low amounts, these proinflammatory cytokines may be protective against viral invasion. If overproduced, however, they may become harmful to the host by mediating an injurious inflammatory response.

West Nile encephalitis

Since 1999, West Nile (WN) virus has been spreading across the entire American continent, killing more than 500 persons. After infection, it causes a spectrum of illnesses, including WN fever, paralysis syndromes, and fetal meningoencephalitis. The pathology of the West Nile (WN) encephalitis includes neuronal degeneration and necrosis, as well as signs of diffuse inflammation. At relative low infectious doses, the WN virus induces apoptotic death of infected cells in vitro (13). At higher infectious doses, however, it induces necrotic cell death caused by profuse budding of WN progeny virus particles (13). Consequently, HMGB1 was passively released into the extracellular space (13,24). If accumulated, HMGB1 may mediate an injurious inflammatory response that contributes to the pathogenesis of West Nile encephalitis. In addition to the WN viruses, other viruses (e.g., salmon anemia virus) can also induce necrotic cell death of infected cells, resulting in concomitant HMGB1 release (24). Taken together, these observations suggest that HMGB1 may be a molecule that allows innate immune cells to respond to viral infection, thereby mediating potentially injurious inflammatory responses (Fig. 3).

SARS

Coronavirus infects alveolar endothelial cells or macrophages, and can induce various proinflammatory cytokines (e.g., IL-1, IL-6, TNF, and IFN- γ). Consequently, SARS patients developed signs of acute lung inflammation including diffuse alveolar damage, airspace edema, and bronchiolar fibrin (4,14,59). Notably, AIDS patients with deficient immune system are somewhat resistant to SARS infection (32), raising a possibility that an excessive immune response is attributable to the lethality of patients who die of SARS (7,32,35,40).

Although the potential role HMGB1 in the pathogenesis of SARS remains elusive, the elevation of proinflammatory cytokines (e.g., IL-1, IL-6, TNF, and IFN- γ) in SARS patients (4,14,59) may trigger active HMGB1 release from innate immune cells. In addition, HMGB1 can be passively released if alveolar endothelial cells or macrophages are damaged by virus-mediated cytolysis (Fig. 3). Once released, extracellular HMGB1 may mediate an injurious pulmonary inflammatory response including neutrophil infiltration, derangement of epithelial barrier, lung edema, and lung injury (7). Collectively, these injurious pulmonary inflammatory responses may consequently lead to respiratory failure, and death.

Hepatitis

Acute viral hepatitis is caused by hepatitis (A, B, C) viruses, and its pathogenesis is characterized by acute necrosis of hepatocytes, inflammation, and subsequent fibrosis and

cirrhosis (41). Like many other viruses, hepatitis viruses themselves may not be directly cytopathic, but can trigger an inflammatory response as manifested by the production of various proinflammatory cytokines (17,18,23). If overproduced, these proinflammatory cytokines may cause self-inflicted hepatocellular injury in infected patients (17,18,23). Consequently, HMGB1 may be passively released from necrotic hepatocytes, and activates tissue macrophage (Kupffer cells) to release proinflammatory cytokines (Fig. 3). Thus HMGB1, by itself or in combination with other proinflammatory cytokines, may contribute to the persistent liver injury in hepatitis patients.

Several anti-inflammatory agents (e.g., IL-10, or TNF antagonists) have shown efficacy in reducing liver inflammatory injury (23), but adversely augment viral titers in some hepatitis patients (23), confirming a dual role for proinflammatory cytokines in the pathogenesis of hepatitis. In contrast, a phase II clinical trial using IFN- γ , an important proinflammatory cytokine against viral infections (12), failed in the treatment of liver fibrosis (<http://www.intermune.com/wt/itmn/corp>). It remains elusive whether this failure relates to the capacity of IFN- γ to induce a number of a potentially injurious proinflammatory cytokines (such as TNF and HMGB1) (42).

Influenza

Influenza (also known as the “flu”) is caused by influenza (types A, B, and C) viruses and is characterized by massive virus replication and excessive inflammation. In addition to epithelial cells of the upper respiratory system, influenza viruses also infect monocytes/macrophages, and induce various proinflammatory cytokines (e.g., TNF, IL-1, IL-6, IL-8, IFN- α , and chemokines) in infected regions (25,51). Influenza viruses destroy infected cells via apoptosis near the end of the replication cycle to prevent setting off an inflammatory response. However, infected monocytes/macrophages can release various proinflammatory cytokines (25), which could induce active HMGB1 release (Fig. 3). Therefore, it will be interesting to investigate whether HMGB1 plays a role in the pathogenesis of influenza in future studies.

Influenza A viruses continuously mutate their surface coat proteins (i.e., hemagglutinin and neuraminidase [NA]) by point mutation (“antigenic drift”) or whole-gene substitution (“antigenic shift”) to give rise to novel viruses that can escape from the acquired immunity of the host. Consequently, an epidemic of a mutated influenza A (H1N1) killed more than 40 million persons during the period 1918–1919. Recently, influenza A (H5N1) viruses have been spreading among poultry in Asia since 2003. Unlike other human, avian, and swine influenza viruses, these H5N1 influenza viruses are resistant to the antiviral effects of TNF and IFNs (45). If these H5N1 viruses become infectious to human beings after dramatic antigenic mutations, they could be destructive because human beings lack acquired immunity against these novel viruses. Thus, future studies are needed to investigate the potential role of HMGB1 in the pathogenesis of influenza and other viral infectious diseases.

CONCLUSION

HMGB1 is released by necrotic cells and activated macrophages/monocytes, and functions as a critical mediator of lethal systemic inflammation. After viral infection, it may be actively or passively released by infected cells, and initiates a pronounced inflammatory response driven both by infected cells and neighboring innate immune cells. It is currently unknown whether HMGB1-mediated inflammatory response contributes to the pathogenesis of various viral infectious diseases. Further investigation in this area may identify novel therapeutic strategies for viral infectious and other inflammatory diseases.

ACKNOWLEDGMENTS

The studies summarized in this review were supported in part by grants (R01GM063075, R01GM070817 to H.W.) from the National Institute of General Medical Science, National Institutes of Health.

REFERENCES

1. Abraham E, Arcaroli J, Carmody A, et al. HMG-1 as a mediator of acute lung inflammation. *J. Immunol* 2000;165:2950–2954. [PubMed: 10975801]
2. Agnello D, Wang H, Yang H, et al. HMGB1, 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. [PubMed: 12126646]
3. 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. [PubMed: 10952726]
4. Bermejo JF, Munoz-Fernandez MA. Severe acute respiratory syndrome, a pathological immune response to the new coronavirus—implications for understanding of pathogenesis, therapy, design of vaccines, and epidemiology. *Viral Immunol* 2004;17:535–544. [PubMed: 15671750]
5. 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. [PubMed: 14532127]
6. Chacur M, Milligan ED, Gazda LS, et al. A new model of sciatic inflammatory neuritis (SIN): induction of unilateral and bilateral mechanical allodynia following acute unilateral peri-sciatic immune activation in rats. *Pain* 2001;94:231–244. [PubMed: 11731060]
7. Chen G, Chen DZ, Li J, et al. Pathogenic role of HMGB1 in SARS? *Med. Hypotheses* 2004;63:691–695. [PubMed: 15325019]
8. Chen G, Li J, Ochani M, et al. Bacterial endotoxin stimulates macrophages to release HMGB1 partly through CD14- and TNF-dependent mechanisms. *J. Leukoc. Biol* 2004;76:994–1001. [PubMed: 15331624]
9. Chen G, Li J, Qiang X, et al. Suppression of HMGB1 release by stearyl lysophosphatidylcholine: an additional mechanism for its therapeutic effects in experimental sepsis. *J. Lipid Res* 2005;46:623–627. [PubMed: 15687351]
10. Chen G, Ward MF, Sama AE, et al. Extracellular HMGB1 as a proinflammatory cytokine. *J. Interferon Cytokine Res* 2004;24:329–333. [PubMed: 15212706]
11. Chen X, Li W, Wang H. More tea for septic patients? Green tea may reduce bacterial endotoxin-induced release of high mobility group box 1 and other proinflammatory cytokines. *Med. Hypotheses* 2006;66:660–663. [PubMed: 16266789]
12. Chesler DA, Reiss CS. The role of IFN-gamma in immune responses to viral infections of the central nervous system. *Cytokine Growth Factor Rev* 2002;13:441–454. [PubMed: 12401479]
13. Chu JJ, Ng ML. The mechanism of cell death during West Nile virus infection is dependent on initial infectious dose. *J. Gen. Virol* 2003;84:3305–3314. [PubMed: 14645911]
14. Clark RK, Lee EV, White RF, et al. Reperfusion following focal stroke hastens inflammation and resolution of ischemic injured tissue. *Brain Res. Bull* 1994;35:387–392. [PubMed: 7850491]
15. Cui X, Wu R, Zhou M, et al. Adrenomedullin and its binding protein attenuate the proinflammatory response after hemorrhage. *Crit. Care Med* 2005;33:391–398. [PubMed: 15699844]
16. Degryse B, Bonaldi T, Scaffidi P, et al. The high mobility group (HMG) boxes of the nuclear protein HMGB1 induce chemotaxis and cytoskeleton reorganization in rat smooth muscle cells. *J. Cell Biol* 2001;152:1197–1206. [PubMed: 11257120]
17. Fan XG, Zhang Z. Increased tumour necrosis factor alpha production by neutrophils in patients with hepatitis B. *J. Clin. Pathol* 1994;47:616–618. [PubMed: 8089217]
18. Fan XG, Liu WE, Li CZ, et al. Circulating Th1 and Th2 cytokines in patients with hepatitis C virus infection. *Mediators Inflamm* 1998;7:295–297. [PubMed: 9792341]
19. Fiuza C, Bustin M, Talwar S, et al. Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. *Blood* 2003;101:2652–2660. [PubMed: 12456506]
20. 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;3:955–1001.

21. 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. [PubMed: 12183440]
22. Huttunen HJ, Rauvala H. Amphoterin as an extracellular regulator of cell motility: from discovery to disease. *J. Intern. Med* 2004;255:351–366. [PubMed: 14871459]
23. Jacobson Brown PM, Neuman MG. Immunopathogenesis of hepatitis C viral infection: Th1/Th2 responses and the role of cytokines. *Clin. Biochem* 2001;34:167–171. [PubMed: 11408013]
24. Joseph T, Cepica A, Brown L, et al. Mechanism of cell death during infectious salmon anemia virus infection is cell type-specific. *J. Gen. Virol* 2004;85:3027–3036. [PubMed: 15448366]
25. Julkunen I, Sareneva T, Pirhonen J, et al. Molecular pathogenesis of influenza A virus infection and virus-induced regulation of cytokine gene expression. *Cytokine Growth Factor Rev* 2001;12:171–180. [PubMed: 11325600]
26. Kim JY, Park JS, Strassheim D, et al. HMGB1 contributes to the development of acute lung injury after hemorrhage. *Am. J. Physiol. Lung Cell Mol. Physiol* 2005;288:L958–L965. [PubMed: 15640285]
27. 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. [PubMed: 15644117]
28. 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. [PubMed: 12765338]
29. Li W, Sama AE, Wang H. Role of HMGB1 in cardiovascular diseases. *Curr. Opin. Pharmacol* 2006;6:130–135. [PubMed: 16487750]
30. Limana F, Germani A, Zacheo A, et al. Exogenous high-mobility group box 1 protein induces myocardial regeneration after infarction via enhanced cardiac C-kit+ cell proliferation and differentiation. *Circ. Res* 2005;97:e73–e83. [PubMed: 16166556]
31. Lin X, Yang H, Sakuragi T, et al. α -Chemokine receptor blockade reduces high mobility group box 1 protein-induced lung inflammation and injury and improves survival in sepsis. *Am. J. Physiol. Lung Cell Mol. Physiol* 2005;289:L583–L590. [PubMed: 15937067]
32. Mandavilli A. Immune response to SARS sets up puzzling paradox. *Nat. Med* 2004;10:1268. [PubMed: 15580237]
33. 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. [PubMed: 15210788]
34. Muller S, Ronfani L, Bianchi ME. Regulated expression and subcellular localization of HMGB1, a chromatin protein with a cytokine function. *J. Intern. Med* 2004;255:332–343. [PubMed: 14871457]
35. Nicholls JM, Poon LL, Lee KC, et al. Lung pathology of fatal severe acute respiratory syndrome. *Lancet* 2003;361:1773–1778. [PubMed: 12781536]
36. Ombrellino M, Wang H, Ajemian MS, et al. Increased serum concentrations of high-mobility-group protein 1 in haemorrhagic shock [letter]. *Lancet* 1999;354:1446–1447. [PubMed: 10543678]
37. Palumbo R, Sampaioles M, De Marchis F, et al. Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. *J. Cell. Biol* 2004;164:441–449. [PubMed: 14744997]
38. Park JS, Arcaroli J, Yum HK, et al. Activation of gene expression in human neutrophils by high mobility group box 1 protein. *Am. J. Physiol. Cell. Physiol* 2003;284:C870–C879. [PubMed: 12620891]
39. Park JS, Svetkauskaite D, He Q, et al. Involvement of TLR 2 and TLR 4 in cellular activation by high mobility group box 1 protein (HMGB1). *J. Biol. Chem* 2003;279:7370–7377. [PubMed: 14660645]
40. Poon LL, Guan Y, Nicholls JM, et al. The aetiology, origins, and diagnosis of severe acute respiratory syndrome. *Lancet Infect. Dis* 2004;4:663–671. [PubMed: 15522678]
41. Popper H. Clinical pathologic correlation in viral hepatitis. The effect of the virus on the liver. *Am. J. Pathol* 1975;81:609–628. [PubMed: 1108667]
42. 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. [PubMed: 12646658]

43. Sappington PL, Yang R, Yang H, et al. HMGB1 B box increases the permeability of Caco-2 enterocytic monolayers and causes derangements in intestinal barrier function in mice. *Gastroenterology* 2002;123:790–802. [PubMed: 12198705]
44. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002;418:191–195. [PubMed: 12110890]
45. Seo SH, Hoffmann E, Webster RG. Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. *Nat. Med* 2002;8:950–954. [PubMed: 12195436]
46. Sunden-Cullberg J, Norrby-Teglund A, Rouhiainen A, et al. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. *Crit. Care Med* 2005;33:564–573. [PubMed: 15753748]
47. 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. [PubMed: 12974876]
48. 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. [PubMed: 15795240]
49. Ueno H, Matsuda T, Hashimoto S, et al. Contributions of high mobility group box protein in experimental and clinical acute lung injury. *Am. J. Respir. Crit. Care Med* 2004;170:1310–1316. [PubMed: 15374839]
50. 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. USA* 2002;99:12351–12356. [PubMed: 12209006]
51. Van Reeth K. Cytokines in the pathogenesis of influenza. *Vet. Microbiol* 2000;74:109–116. [PubMed: 10799783]
52. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999;285:248–251. [PubMed: 10398600]
53. Wang H, Czura CJ, Tracey KJ. Lipid unites disparate syndromes of sepsis. *Nat. Med* 2004;10:124–125. [PubMed: 14760415]
54. Wang H, Li W, Li W, J, et al. The aqueous extract of a popular herbal nutrient supplement, *Angelica sinensis*, protects mice against lethal endotoxemia and sepsis. *J. Nutr* 2006;136:360–365. [PubMed: 16424112]
55. Wang H, Liao H, Ochani M, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat. Med* 2004;10:1216–1221. [PubMed: 15502843]
56. 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. [PubMed: 10455911]
57. Wang H, Yang H, Czura CJ, et al. HMGB1 as a late mediator of lethal systemic inflammation. *Am. J. Respir. Crit. Care Med* 2001;164:1768–1773. [PubMed: 11734424]
58. Wang H, Yang H, Tracey KJ. Extracellular role of HMGB1 in inflammation and sepsis. *J. Intern. Med* 2004;255:320–331. [PubMed: 14871456]
59. Ward SE, Loutfy MR, Blatt LM, et al. Dynamic changes in clinical features and cytokine/chemokine responses in SARS patients treated with interferon alfacon-1 plus corticosteroids. *Antivir. Ther* 2005;10:263–275. [PubMed: 15865221]
60. Yan JJ, Jung JS, Lee JE, et al. Therapeutic effects of lysophosphatidylcholine in experimental sepsis. *Nat. Med* 2004;10:161–167. [PubMed: 14716308]
61. 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. [PubMed: 14695889]
62. 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. [PubMed: 16205327]


```

1  MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWK 50
      "A Box"
51  TMSAKEKGKFEDMAKADKARYEREMKTYIPPKGETKKKFKDPNAPKRPPS 100
101 AFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTAADDKQPYEKKA 150
      "Cytokine Domain"      "B Box"
151 LKEKYEKDIAAYRAKGGKPDAAKGGVVKAEKSKKKKEEEDEEDEE 200
      "RAGE-binding"
201 EDEEDEDDEEDDDDE 215

```

FIG. 1.

Amino acid sequence of human high mobility group box 1 (HMGB1). The C-terminal portion of HMGB1 contains an acidic tail, and the N-terminal portion of HMGB1 comprises two internal repeats of a positively charged domain of about 80 amino acids (termed "HMG boxes") (shown by bold text). The cytokine-stimulating motif ("Cytokine Domain") of HMGB1 does not overlap with its receptor for advanced glycation end products (RAGE)-binding site, implicating the potential involvement of other cell surface receptors for HMGB1-mediated inflammatory responses.

FIG. 2. Cytoplasmic translocation of high mobility group box 1 (HMGB1) in activated macrophage cultures. Quiescent macrophages (**panel A**) preserve a pool of pre-made HMGB1 predominantly in the nucleus. In response to various stimuli (e.g., bacterial endotoxin, lipopolysaccharide [LPS]), macrophages actively translocate HMGB1 into the cytoplasm before releasing into the extracellular milieu (**panel B**). (**Upper panels**) Light-translucent pictures to show cellular morphology. (**Lower panels**) Fluorescent images to show HMGB1 immunostaining. Arrows point to nuclear regions of representative cells.

FIG. 3.

Hypothetical roles of high mobility group box 1 (HMGB1) in the pathogenesis of viral infectious diseases. HMGB1 may be actively or passively released by infected cells, and initiates a pronounced inflammatory response driven both by infected cells and neighboring innate immune cells. The viral infection-elicited inflammatory response may contribute to the pathogenesis of viral infectious diseases.