Review Article

Novel insights for high mobility group box 1 protein-mediated cellular immune response in sepsis: A systemic review

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BACKGROUND: High mobility group box 1 protein (HMGB1) is a highly conserved, ubiquitous protein in the nuclei and cytoplasm of nearly all cell types. HMGB1 is secreted into the extracellular milieu and acts as a proinflammatory cytokine. In this article we reviewed briefly the cellular immune response mediated by HMGB1 in inflammation and sepsis.

METHODS: This systemic review is mainly based on our own work and other related reports.

RESULTS: HMGB1 can actively affect the immune functions of many types of cells including T lymphocytes, regulatory T cells (Tregs), dendritic cells (DCs), macrophages, and natural killer cells (NK cells). Various cellular responses can be mediated by HMGB1 which binds to cell-surface receptors [e.g., the receptor for advanced glycation end products (RAGE), Toll-like receptor (TLR)2, and TLR4]. Anti-HMGB1 treatment, such as anti-HMGB1 polyclonal or monoclonal antibodies, inhibitors (e.g., ethyl pyruvate) and antagonists (e.g., A box), can protect against sepsis lethality and give a wider window for the treatment opportunity.

CONCLUSION: HMGB1 is an attractive target for the development of new therapeutic strategies in the treatment of patients with septic complications.

KEY WORDS: High mobility group box 1 protein; Sepsis; Immunological effect; Cytokine; Signal transduction

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INTRODUCTION

Sepsis is a systemic, inflammatory response to infection associated with diffuse coagulopathy, multiple organ dysfunction syndrome (MODS), and even death. The mortality rate from severe sepsis remains over 30% despite advances in intensive care therapy. The annual health care expenditure associated with this ailment is as high as \$16.7 billion per year in the United States.^[1] Previous studies^[2,3] on the pathogenesis of sepsis has highlighted the importance of proinflammatory mediators in the course of sepsis. Despite success in animals, clinical trials aiming at inhibiting the early cytokine mediators [i.e., tumor necrosis factor (TNF) or interleukin (IL)-1]

have failed to improve survival in septic patients.^[4,5] An alternative approach is to target other later mediators in the pathogenesis of sepsis in order to provide a wider window of opportunity for the treatment of this lethal syndrome.

In 1999, studies^[6,7] revealed that high mobility group box 1 protein (HMGB1) was released by cultured macrophages 8 hours after stimulation with endotoxin, TNF- α , or IL-1. It was also found that once released into the extracellular milieu, HMGB1 activates inflammatory responses.^[8] Furthermore, delayed administration of antibodies to HMGB1 attenuated endotoxin lethality in mice, while administration of HMGB1 alone was lethal.

During the past 10 years, a series of basic scientific observations focused on HMGB1 have indicated that this protein can exert profound influence on multiple systems of the host with severe sepsis or trauma, including inflammation, immune system, blood coagulation system, etc.^[9-11] These findings suggest that HMGB1 is unique in its delayed release kinetics and that it could be an important therapeutic target in lethal systemic inflammatory diseases in which excessive amounts of HMGB1 are released.

This review summarizes the recent advances in investigating the cytokine role of HMGB1 in mediating inflammatory diseases and sepsis, its interactions with other types of immunological cells, and its putative signal transduction pathways, including interaction with cell-surface receptors and intracellular signaling. Finally, the therapeutic potential of blocking HMGB1 activity in the treatment of septic complications is discussed.

HMGB1-mediated cellular immune effect on sepsis

Sepsis and associated diseases such as systemic inflammatory response syndrome (SIRS) and MODS represent common posttraumatic complications in intensive care units induced by a variety of body defense mechanisms. Cytokines have been defined as proteins that can be released from activated immunocytes and mediate diverse immunological responses in sepsis.^[12] Several laboratories have demonstrated independently that HMGB1, a ubiquitous and chromosomal protein, can actively affect the immune functions of a number of cells including T lymphocytes,^[13] regulatory T cells (Tregs),^[14] dendritic cells (DCs),^[15] macrophages,^[16] and natural killer cells (NK cells).^[17] Once released, HMGB1 can bind to cell-surface receptors and mediate various cellular responses including chemotactic cell movement and release of proinflammatory cytokines (e.g., TNF- α and IL-1).^[18,19] Taken together, these observations characterized HMGB1 as a nonclassical, proinflammatory cytokine.

HMGB1 and T lymphocytes

It is well known that pathogenic microorganism is first recognized by the innate immune system of the host after its invasion. Therefore, T-lymphocyte and antigen presenting cells (APCs) play important roles in cell-mediated immunity. Previous reports^[20,21] have suggested that sepsis or severe trauma mainly causes marked T cell-mediated immune suppression, which contributes to depression of host resistance to infection. Our data also revealed that HMGB1 released after major burns was associated with the immunosuppression in splenic

T-lymphocytes in rats.^[13] Simultaneously, Dong et al^[22] investigated the change in T cell-mediated immunity and its relationship with plasma HMGB1 levels in patients with severe burn injury. The results showed that plasma HMGB1 levels were markedly elevated on post burn day (PBD) 1 in severely burned patients, and it was significantly higher in MODS group than in non-MODS group. On the other hand, lymph proliferation response and IL-2 production of T cells in peripheral blood, and the ratio of CD4⁺/CD8⁺ T cells in the MODS group were markedly lower than those in the non-MODS group on PBD 1, 14, 21 and 28. The findings indicated that plasma HMGB1 levels were negatively correlated to T cellular immune function parameters, including lymphocyte proliferation response, IL-2 production, and the ratio of CD4⁺/CD8⁺ T cells in extensively burned patients. Thus, extensive burns could lead to T cellular immune dysfunction, which appears to be associated with the development of MODS. HMGB1, as an important "late" mediator of inflammation, might be involved in the pathogenesis of suppression of T cell-mediated immunity in these patients. Other data showed that HMGB1 also activates human neutrophils to produce proinflammatory mediators such as TNF- α , IL-1, and IL-8, suggesting an important role of HMGB1 in activation of neutrophils during inflammation.^[23]

HMGB1 and Tregs

Hiraki et al^[24] proposed that Tregs play a central role in the maintenance of immunological tolerance in the peripheral lymphatic system and are also essential for an effective immunosuppressive response to severe sepsis. Some experiments have suggested that tumor cell-derived HMGB1 may suppress naturally acquired CD8⁺ T cell-dependent antitumor immunity via enhancing Tregs to produce IL-10, which is necessary for Tregs-mediated immune depression.^[14] Zhang et al^[25] found that HMGB1 stimulation could result in marked down-regulation of Foxp3 expression, which is considered to be a "master gene" in controlling the development and function of Tregs, as well as secretion of IL-10 from splenic Tregs in mice. Therefore, HMGB1 might be also involved in modulating cell-mediated immunity by influencing proliferation of effector T cells, secretion of IL-2, and cell polarization. Our study further demonstrated that excessive release of HMGB1 could stimulate CD4⁺CD25⁺ Tregs activity via binding advanced glycation end products (RAGE) on the surface of Tregs and trigger a shift of Th1 to Th2 with suppression of T-lymphocyte immune function after major burns. Simultaneously, these effects are partly inhibited

in the presence of ethyl pyruvate (EP), by inhibiting HMGB1 or anti-RAGE antibody.^[26] Nevertheless, our experiment *in vitro* demonstrated that HMGB1 might have a dual regulatory effect on immune functions of Tregs and T cells with different concentrations and stimulation duration.^[25] It is also indicated that efficient inhibition of HMGB1 expression could be a feasible therapeutic strategy in the treatment of organ failure by suppressing endotoxemia and enhancing Treg cell activity.^[27]

HMGB1 and DCs

DCs are key components of innate and adaptive immune responses. The mobilization of DCs from peripheral tissues is critical for the establishment of T cell-dependent immune responses or tolerance, because the physical interaction of DCs with naïve T cells takes place in the T cell areas of lymph nodes.^[28] Chemokines regulate the migration and the maturation of DCs licensed by microbial constituents. It has been recently found that the function of DCs, including their ability to activate naïve, allogeneic CD4⁺ T cells, requires the autocrine/paracrine release of the nuclear protein HMGB1.^[15] HMGB1 acts as a chemoattractant and activator of DCs. HMGB1 induced the migration of monocyte-derived, immature DCs (Mo-iDCs) but not mature DCs. The chemotactic effect of HMGB1 on iDCs was shown to be inhibited by pertussis toxin and down-regulated by antibody against the receptor of RAGE, suggesting that HMGB1 chemoattraction of iDCs is mediated by RAGE in a Gi protein-dependent manner. In addition, HMGB1 treatment of Mo-iDCs up-regulated DCs surface markers (CD80, CD83, CD86, and HLA-A, B, C), enhanced DC production of a number of cytokines (IL-6, CXCL8, IL-12p70, and TNF- α), switched DCs chemokine responsiveness from CCL5-sensitive to CCL21-sensitive, and acquired the capacity to stimulate allogeneic T cell proliferation. Based on its dual DC-attracting and -activating activities as well as its reported capacity to promote an antigen-specific immune response, it is considered that HMGB1 has the properties of an immune alarmin.^[29] Furthermore, HMGB1 may direct the inflammatory responses mediated by DCs, at least in part, by enhancing Toll-like receptor 4 (TLR4) expression and reactivity to it and other damage-associated molecular pattern molecules (DAMPs).^[30]

Zhang et al^[31] investigated the effect of HMGB1 on the maturation of DCs *in vivo* and the influence on T-cell-mediated immunity in rats after thermal injury. The results showed that the expression levels of splenic HMGB1 were significantly elevated during PBD 1–7. DCs expressed similar levels of CD80, strongly enhanced

CD86, and slightly elevated MHC class II levels in comparison to DCs obtained from sham-injured rats, but protein levels of IL-12 were not increased after thermal injury. Administration of EP to inhibit HMGB1 could significantly enhance expression levels of CD80, MHC class II on DCs surface, and IL-12 production after burns. Concomitantly, proliferative activity and expression levels of IL-2 as well as IL-2R α of T cells were restored. These results implied that the excessively released HMGB1 might stimulate splenic DCs to mature abnormally and down-regulate the IL-12 production, and further shifting of Th1 to Th2 with suppression of T-lymphocyte immune function following burn injury. Recently, our experiment also demonstrated an important role of endoplasmic reticulum stress (ERS) and its regulator XBP-1 in HMGB1-induced maturation and activation of DCs.^[32] In addition, HMGB1 was noted to induce the differentiation of splenic DCs to CD11c^{low}CD45RB^{high} DCs followed by shifting of Th1 to Th2 with enhancement of T-lymphocyte immune function *in vitro*. However, the effect of HMGB1 on T cell differentiation to Th2 was not associated with the inhibition of IL-12 production in CD11c^{high}CD45RB^{low} DCs.^[33]

HMGB1 and macrophages

Phagocytosis of apoptotic cells is critical for resolution of inflammation. HMGB1 has been shown to diminish phagocytosis through binding to phosphatidylserine (PS) exposed on the surface of apoptotic neutrophils.^[34] However, it is currently unknown whether HMGB1 also modulates the activity of receptors involved in PS recognition on the surface of macrophages. Friggeri et al^[16] found that preincubation of macrophages with HMGB1 decreased their ability to engulf apoptotic neutrophils or thymocytes. Preincubation of macrophages with HMGB1 prevented the enhancement of efferocytosis resulting from exposure to milk fat globule EGF factor 8 (MFG-E8), which was an opsonin that bridges PS and $\alpha(v)\beta(3)$ as well as $\alpha(v)\beta(5)$ -integrins on the surface of phagocytes. HMGB1 also inhibited intracellular signaling events, including extracellular regulated protein kinases (ERK) phosphorylation and Rac-1 activation, which are activated in macrophages during phagocytosis of apoptotic cells. These results indicate that HMGB1 blocks $\alpha(v)\beta(3)$ -dependent recognition and uptake of apoptotic cells.

In cultured human primary macrophages/monocytes, HMGB1 is potent in stimulating the release of multiple proinflammatory cytokines, including TNF- α , IL-1 α , IL-1 β , IL-1ra, IL-6, IL-8, MIP-1 α , and MIP-1 β , but not IL-10 and IL-12.^[35] The kinetics of HMGB1-or

endotoxin-induced TNF- α release is notably different, as HMGB1 induces TNF- α release significantly later than endotoxin, which induces the release of TNF- α in a single peak 2-3 hours after stimulation, whereas HMGB1 induces a biphasic TNF- α response, with the first peak at approximately 3 hours and a second peak 8–10 hours after HMGB1 exposure. Recently, Kawahara et al^[36] demonstrated that C-reactive protein, a prominent risk marker for inflammation, including atherosclerosis, could induce the active release of HMGB1 by macrophages through Fc gamma receptor/p38 mitogen-activated protein kinase (MAPK) signaling pathways.

HMGB1 and NK cells

NK cells are a group of cells participating in the innate immune system. They are thought to play an important role in the development of such syndromes resulting from interplay with other types of immune cells ending in subsequent activation of the inflammatory cascade. Thus, depletion of NK cells results in attenuation of inflammatory reaction and an overall improvement in outcome.^[37] Therefore, NK cells can be considered as important targets for therapeutic strategies. Depletion of NK cells in a murine polytrauma model is associated with improved outcome and a modulation of the inflammatory response.^[38] Gougeon et al^[17] found that HMGB1 enhanced interferon gamma release from macrophage (but not dendritic cell)-stimulated NK cells. This is effective only when coupled with other proinflammatory cytokines particularly with IL-2 in combination with IL-1 or IL-12.

Interaction of NK cells with autologous iDCs results in reciprocal activation. A study^[39] reported that NK cells triggered iDCs to polarize and secrete IL-18, in turn, DC-activated NK cells secreted the nuclear protein/proinflammatory cytokine HMGB1, which induces DCs maturation and prevents DCs from lysis. However, activated NK cells can also kill iDCs. To investigate whether effector and maturative properties may coexist or segregate in different NK subsets, human NK cell clones were generated and analyzed for their effects on iDCs. Semino et al^[40] noticed that the ability of different NK cell clones to induce iDCs maturation was unlinked to their phenotypic and cytolytic features but correlated with the relocation of HMGB1 from nucleus to cytoplasm. "Maturative" NK cell clones secrete HMGB1 spontaneously. It is interesting that secretion is strongly enhanced by engagement of the surface molecule NKp30 but only slightly induced by triggering of the activating NK receptor CD16. However, culturing freshly isolated NK cells for 1 week with low doses of anti-CD16 triggers

the relocation of HMGB1 from nucleus to cytoplasm and its spontaneous secretion, thus resulting in a stronger maturation potential of the NK cells. Taken together, these data indicate that NK cells comprise functionally different subsets which are endowed with different capacities to secrete HMGB1 and to induce maturation of autologous iDCs.

Receptor(s) for HMGB1 and related intracellular signaling in sepsis

The inflammatory properties of HMGB1 depend at least partially on the ability to combine with soluble moieties, including nucleic acids, microbial products, and cytokines.^[41] Meanwhile, intracellular receptors are also important to the proinflammatory effect of HMGB1. To date, eight candidate receptors have been implicated in mediating the biological responses to HMGB1.^[42] As a receptor of multiple ligands, RAGE has been implicated as a critical receptor mediating the cytokine activity of HMGB1 in Tregs and macrophages.^[25,43] Interaction of RAGE with ligand has two main consequences: one is to activate CDC42, Rac, and guanosine triphosphatases that regulate cell motility, and the other pathway activates several MAPKs and subsequently leads to activation of nuclear factor (NF)- κ B.^[44] Interaction of RAGE and HMGB1 causes phosphorylation of MAPKs (e.g., p38 and p42/44 kinases, stress-activated protein kinase/c-Jun N-terminal kinase, ERK1/2) and activation of the NF- κ B signaling pathway in cultured macrophages, neutrophils, and Caco-2 epithelial cells.^[45,46] Moreover, HMGB1-mediated smooth muscle cell migration involves activation of MAPK pathways and a G-protein-coupled receptor.^[47] RAGE also plays a nonredundant role in DCs homing to lymph nodes. Thus, HMGB1 promotes the up-regulation of receptors for lymph node chemokines, regulates the remodeling of the cytoskeleton of migrating cells, and sustains their journey to secondary lymphoid organs via a RAGE-dependent pathway.^[48] The HMGB1-RAGE pathway is a checkpoint in DCs maturation and function and a candidate for targeted therapies.^[49]

Nonetheless, current evidences suggest that RAGE alone could not explain all the effects of HMGB1, as discrepancies exist between specific HMGB1-binding/RAGE activation and biological effects of HMGB1. Recent data indicate that TLR4, a pivotal receptor for activation of innate immunity and cytokine release, may also play a role in HMGB1 signaling.^[50] Documents show that TLR4 is required for HMGB1-dependent activation of macrophage and TNF- α release. Surface plasmon resonance studies indicate that HMGB1 binds

specifically to TLR4, and that this binding requires a cysteine in position 106. Inhibition of TLR4 binding with neutralizing anti-HMGB1 monoclonal antibody or by mutating cysteine 106 prevents HMGB1 activation of cytokine release.^[51] Our results also suggested that TLR4 might be a potential receptor essential for the negative effect of HMGB1 on CD4⁺CD25⁺ Tregs activity.^[52] These results have implications for rationale, design, and development of experimental therapeutics for use in non-infectious and infectious inflammation.^[51] Using human embryonic kidney cells overexpressing TLR2 or TLR4, it was found that HMGB1 induced IL-8 release only from TLR2-overexpressing cells and not from TLR4 or control vector-overexpressing cells. Furthermore, no bacterial TLR2 agonists (peptidoglycan-associated lipoprotein, antimurein lipoprotein, outer membrane protein A) were detected in HMGB1 proteins. Although the interaction of RAGE, TLR2, and TLR4 and the relative contributions of different receptors to HMGB1 signaling are still under investigation, results to date indicate that RAGE and members of the TLRs are important receptors in HMGB1 signaling. Therefore, antagonists of HMGB1/RAGE/TLRs might have therapeutic potential for the treatment of systemic septic diseases.^[53]

Anti-HMGB1 therapies of sepsis

A growing body of studies have demonstrated that HMGB1, released passively by necrotic or damaged cells or actively by macrophages/monocytes, causes inflammatory responses and sepsis.^[6,10–11] Passive immunization with anti-HMGB1 antibodies significantly protected against lethal endotoxemia in mice, even when treatment was delayed 2 hours after endotoxin exposure.^[54] The effects of anti-HMGB1 antibodies were dose-dependent and were effective even after the peak of circulating TNF- α was resolved.^[6] Similar protective effects were observed with other anti-HMGB1 treatments by A box, a DNA-binding motif of HMGB1 and a specific HMGB1 antagonist,^[54] or EP, a nontoxic food additive and an experimental anti-inflammatory agent.^[55] New HMGB1 protein was synthesized in the nucleus and transferred into the cytoplasm, causing an increase in HMGB1 in the nucleus and cytoplasm. EP inhibits HMGB1 mRNA up-regulation and release from endotoxin-stimulated macrophages. The molecular function of EP is to attenuate the activation p38 MAPK, NF- κ B, and CBP signaling pathways.^[56,57] Besides endotoxemia, the protection conferred by anti-HMGB1 treatment also applies to other models of inflammation. Delayed treatment with anti-HMGB1 antibodies or other antagonists (A box or EP)

dose-dependently rescued mice from lethal sepsis induced by cecal perforation, and the treatment was effective even when the first dose was given 24 hours after the cecal ligation and puncture procedure.^[54,55] Recently developed anti-HMGB1 monoclonal antibodies confirmed these findings.^[58] *In vitro* studies showed that A box competitively inhibited ¹²⁵I-labeled HMGB1 cell-surface binding and attenuated HMGB1-induced proinflammatory cytokine release in macrophage-like RAW 264.7 cells,^[54] and EP specifically decreased endotoxin-induced HMGB1 release and down-regulated p38 MAPK and NF- κ B activation in macrophage cultures.^[55] Thus, anti-HMGB1 treatment with HMGB1 antibodies, specific antagonist A box, or anti-inflammatory agent EP can rescue mice from lethal, systemic inflammation, or even the treatment was delayed (2 hours after endotoxin administration and 24 hours after cecal ligation and puncture). It is thus feasible to develop HMGB1-targeted, therapeutic strategies for the clinical management of lethal systemic inflammatory diseases.

CONCLUSION

Severe sepsis represents one of the most common disease entities in patients admitted to the intensive care unit. Despite modern advances in critical care, effective treatment of sepsis is still lacking nowadays. The discovery of HMGB1 as a proinflammatory cytokine has initiated a new field of investigation on the development of therapeutics of sepsis. However, many problems remain to be solved, including clarification of the mechanisms of HMGB1 release from cells, cell-surface receptors, downstream signal transduction pathways and conformation of the most effective strategies for targeting the effect of this mediator in sepsis. Related investigations of the above problems might further help our understanding of the various activities of HMGB1, and it might eventually lead to the development of specific and timely therapeutic agents that can reduce the high morbidity and mortality of patients with severe sepsis. Hopefully, this anticytokine-based therapy will be evaluated soon in clinical trials of inflammatory and septic diseases, in which an excessive amount of HMGB1 is produced.

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