HMGB1: A multifunctional alarmin driving autoimmune and inflammatory disease

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Abstract | HMGB1 is a non-histone nuclear protein that can serve as an alarmin to drive the pathogenesis of inflammatory and autoimmune disease. Although primarily located in the cell nucleus, HMGB1 can translocate to the cytoplasm, as well as the extracellular space, during cell activation and cell death; during activation, HMGB1 can undergo post-translational modifications. The activity of HMGB1 varies with the redox states of the cysteine residues, which are required for binding to TLR4. In addition to stimulating cells directly, HMGB1 can form immunostimulatory complexes with cytokines and other endogenous and exogenous factors. In the synovia of patients with rheumatoid arthritis, as well as animal models of this disease, extranuclear expression of HMGB1 is increased and blockade of HMGB1 expression attenuates disease in animal models. In systemic lupus erythematosus, HMGB1 can be a component of immune complexes containing anti-DNA because of its interaction with DNA. In myositis, expression of HMGB1 is enhanced in inflamed muscle and can perturb muscle function. Together, these findings indicate that HMGB1 might be an important mediator and biomarker in rheumatic diseases as well as a target of new therapy.

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Introduction

The biological activity of the non-histone nuclear protein HMGB1 (high mobility group protein B1) depends on its location, context and post-translational modification. Inside the cell, HMGB1 binds DNA and modulates chromosomal architecture; this location is not fixed, however, and, with cell activation, injury or death, HMGB1 can translocate outside of the cell. There, HMGB1 can serve as a damage-associated molecular pattern (DAMP) or alarmin to stimulate the innate immune system either by itself or as part of complexes with cytokines, as well as other exogenous and endogenous molecules.[1] This Review summarizes the immunological properties of HMGB1 and its role as a mediator of inflammation in rheumatic disease, as well as a biomarker for key events in pathogenesis.

Molecular biology of HMGB1

HMGB1 is a DNA-binding protein that is abundant in the cell nucleus, although it can also be cytoplasmic (Figure 1). As a nuclear molecule, HMGB1 regulates transcription, repair and recombination through exerting effects on chromosomal architecture.[2] As HMGB1 does not bind tightly to DNA, including promoter sequences, its effect on transcription seems to reflect interactions with both DNA as well as transcription factors. Indeed, HMGB1 can target multiple immunologically relevant systems including p53, nuclear factor (NF)κB, the glucocorticoid receptor and HIV.[3–6] Furthermore, HMGB1 has a role in V(D)J recombination—a mechanism of genetic recombination that is important in the generation of the immunoglobulin and T-cell receptor components of the immune system.[7]

The capacity for HMGB1 to undergo multiple molecular contacts might thus be essential for the protein to act as both an intracellular and extracellular mediator. Consistent with an important role for its transcription, deficiency is lethal: newborn mice with a gene knockout die of hypoglycemia within 24 h, as a result of impaired transcription of genes regulated by the glucocorticoid receptor.[8] Cell lines lacking HMGB1 can nevertheless survive *in vitro*, which suggests that effects on transcription are cell-type specific.[8]

Cellular location

Although predominantly located in the nucleus, HMGB1 can also translocate to the cytoplasm, as well as the extracellular space. This process occurs in several distinct settings: activation of macrophages and other immune cells by cytokines and toll-like receptor (TLR) ligands; necrosis; apoptosis; and hypoxia and ischemia/reperfusion injury of parenchymal cells. Depending on the inducing stimulus, the physical and/or chemical form of HMGB1 can vary. Acetylation of key lysine residues during cell activation alters the nuclear localization and signals migration to the cytoplasm causing the protein to be concentrated into vesicles for eventual secretion.[9–15] As HMGB1 lacks a leader signal sequence (required for transport through the classical secretory pathway), secretion during cell activation represents a non-classical process determined by the vesicular location. In this mechanism, HMGB1 secretion occurs with fusion of vesicles with the plasma

Competing interests
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Key points

- HMGB1 is a non-histone protein that has key roles inside and outside the cell; inside the cell, HMGB1 can bind to DNA as an architectural element
- During activation and cell death, HMGB1 can translocate from the nucleus to the extracellular space; extracellular HMGB1 serves as an alarmin to mediate inflammation
- Depending on its redox state, HMGB1 can stimulate cells through a variety of receptors, including Toll-like receptors, acting alone or in complex with other immune stimulants
- Extracellular HMGB1 levels are elevated in patients with inflammatory and autoimmune disease
- Studies in animals using HMGB1 antagonists support the notion that they should be a target of therapy in various autoimmune and inflammatory diseases

membrane after an inflammatory signal. In this regard, during activation of immune cells by the NLRP3 inflammasome, caspase 1 might have an important role in the extracellular release of HMGB1.[16–18]

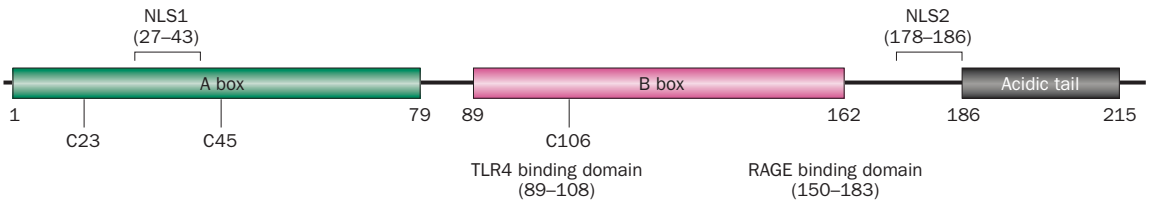
As the immune properties of necrotic and apoptotic cells can differ, the deposition of HMGB1 during cell death is an important issue.[19–21] With necrosis, HMGB1 release occurs passively as cell permeability breaks down.[10,12] The situation with apoptosis is more complicated.[13,14] Initial studies indicated nuclear retention of HMGB1 during apoptosis because of post-translational modifications that affect chromatin binding.[10] Nevertheless, during late apoptosis (secondary necrosis), changes in cell permeability as well as extensive nucleosomal degradation might result in the extracellular release of HMGB1 (Figure 2).

Detection

Quantitating HMGB1 levels in biological fluids is key to exploring the mechanisms of its translocation, as well as its role in physiological and pathological immune responses. In the past, the measurement of HMGB1 levels was hampered by the lack of well-validated and commercially available detection kits. In-house methods such as Western blotting (with or without immunoprecipitation), binding of HMGB1 to hemicatenated DNA or immunochemical staining of tissue samples have been commonly used, although all are time-consuming and difficult for use in large sample cohorts.[1,10,22,23] A commercial ELISA-based method for HMGB1 detection has now become available, which seems to be particularly successful with tissue culture samples and has the potential for screening large numbers of samples. This ELISA is sensitive to levels of HMGB1 at 0.3 μg/l and was used to measure HMGB1 as a prognostic biomarker in sera from patients with acute coronary syndromes.[24]

Irrespective of the method used to detect HMGB1, proteins that bind it can influence assay performance. An example of this situation relates to the detection of HMGB1 in the blood of patients with systemic lupus erythematosus (SLE), where anti-HMGB1 autoantibodies occur with high frequency. Depending on the assay used (that is, immunoblot or ELISA), the values measured for HMGB1 levels can differ markedly as the binding of autoantibodies can prevent detection of the protein by ELISA.[25–27] Furthermore, given the enhancing effects of HMGB1 in complexes with other immunoactive molecules, measuring both free HMGB1 and HMGB1 complexes (including antibodies) is likely to be important in understanding its immunological role.

Current assays for HMGB1 detection do not distinguish between the different forms of HMGB1 that depend on post-translational modification and redox reactions. At present, the only method for such characterization is liquid chromatography-tandem mass spectrometry analysis—a sophisticated and sensitive, yet time-consuming, methodology, which is not well suited for analysis of multiple samples.[28]



1	MGKGDPKKPR	GKMSSYAFFV	QTCREEHKKK	HPDASVNFSE	FSKKCSERWK	TMSAKEKGKF
61	EDMAKADKAR	YEREMKTYIP	PKGETKKKFK	DPNAPKRPPS	AFFLFCSEYR	PKIKGEHPGL
121	SIGDVAKKLG	EMWNNTAADD	KQPYEKKAAK	LKEKYEKDIA	AYRAKGKPDA	AKKGVVKAEK
181	SKKKKEEEED	EEDEEDEEEE	EDEEDEDEEE	DDDDE		

Figure 1 | The structure and function of HMGB1. **a** | The structure of HMGB1, denoting its two DNA binding domains (the A and B boxes) and the acidic C-terminal tail. Also highlighted are the three cysteine residues important for immunological functions. When HMGB1 is released from necrotic cells Cys106 is reduced, whereas, when released from apoptotic cells, it is oxidized. The reduced form of Cys106 is required for the binding of HMGB1 to TLR4 and for its intrinsic cytokine-inducing capacity. A disulfide bond between Cys23 and Cys45 is also required for interaction of HMGB1 with TLR4. HMGB1 residues 150–183 interact with RAGE to mediate chemotaxis, proliferation and differentiation. Intracellular shuttling of HMGB1 between the cytoplasm and nucleus is regulated by the acetylation of lysine residues in the NLS. Reflecting its domain structure, HMGB1 can bind DNA, showing preference for unusual structures such as bends. **b** | The sequence of human HMGB1 with the color-coding of the amino acids corresponding to the functional domains. HMGB1 is highly conserved; the only difference between human HMGB1 and mouse/rat HMGB1 is related to the substitution of Glu189 and Asp202 in human HMGB1 to Asp189 and Glu202 in mouse and rat HMGB1. Abbreviations: NLS, nuclear localization signal domains; RAGE, receptor for advanced glycation end products.

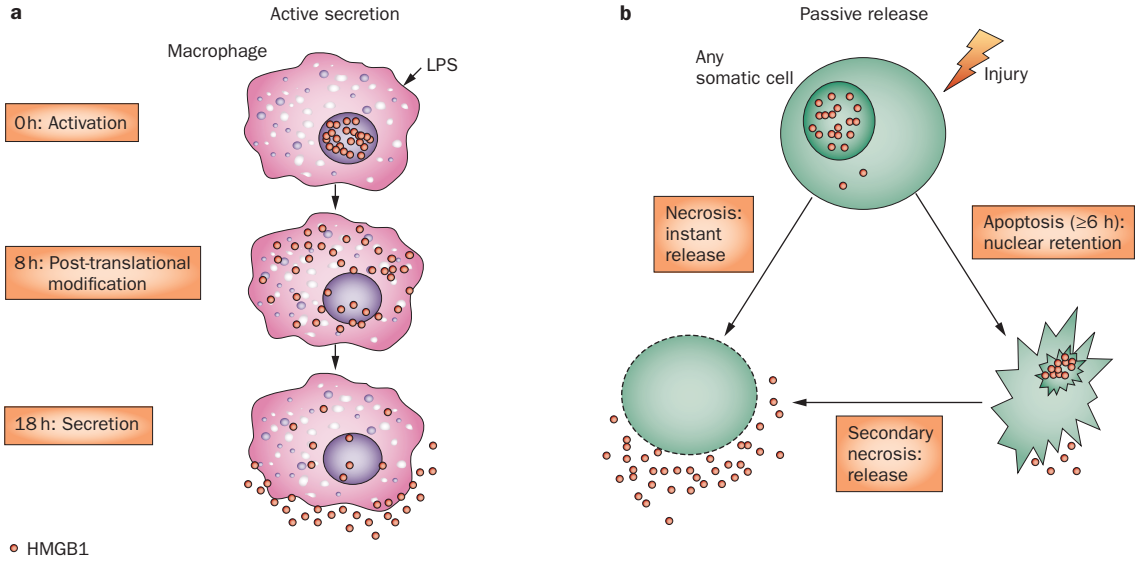


Figure 2 | The extracellular release of HMGB1. HMGB1 is released from cells via multiple pathways. This translocation can represent **a** | an active process in stimulated macrophages and is time-consuming, involving post-translational modification and eventual secretion. **b** | During primary necrosis, HMGB1 release occurs passively and rapidly. During apoptosis, HMGB1 is initially retained in the nucleus but, during secondary necrosis, it can be released passively from apoptotic cells or cell fragments. Abbreviation: LPS, lipopolysaccharide.

At present, many aspects of the biology of extracellular HMGB1 are not well understood, including its stability, its metabolism and the biological activity of any breakdown products that might be generated from intracellular or extracellular proteolytic cleavage. Furthermore, the fates of different forms of HMGB1 are unknown, although post-translational modification and redox state might affect this issue. In this regard, HMGB1 is only one of many intracellular molecules that, once released from cells during activation and death processes, displays immunological activities. Each of these molecules might have its own biology, with studies indicating, for example, that the release of cyclophilin A (another protein with immune activity) differs in its time course from that of HMGB1 during cell death.[29–31]

HMGB1 signaling and inflammation

As now recognized, HMGB1 has a broad repertoire of immunological activities that encompass induction of cytokine production, cell proliferation, chemotaxis, angiogenesis and cell differentiation.[1] In addition to effects on immune cells, HMGB1 can modulate the activities of hematopoietic, epithelial and neuronal cells and mediate systemic effects such as fever, anorexia, and acute-phase responses (Supplementary Table 1). These activities reflect its function as an alarmin and its ability to engage diverse receptors including TLR2, TLR4, TLR9, RAGE (receptor for advanced glycation end products) and CD24–Siglec-10 (Siglec-G in mice).[32–35] In these interactions, post-translational modifications of HMGB1, including acetylation, phosphorylation, methylation and redox changes of cysteine residues, can influence the receptor interactions and downstream signaling events.[11,36–40]

Of receptors important for HMGB1 activity, RAGE was the first demonstrated binding partner.[32] RAGE is a transmembrane, multiligand member of the immunoglobulin superfamily. As shown *in vitro*, HMGB1 signaling through RAGE mediates: chemotaxis; proliferation and differentiation of immune cells and other cells; and upregulation of cell-surface receptors, including TLR4 and RAGE.[1] The role of this pathway in the induction of cytokines is less certain, however. By contrast, for cytokine induction by HMGB1, TLR4 signaling is strictly required—TLR4-deficient macrophages do not release proinflammatory cytokines in response to HMGB1. Although HMGB1 can trigger the recruitment of inflammatory cells through TLR4, this activity is dependent on its redox state, with activation of TLR4 requiring the reduced form of HMGB1 Cys106; importantly, oxidation of Cys106 prevents this interaction.[38–40] This modification might contribute to differences in the immune activity of necrotic when compared with apoptotic cells. HMGB1 released from necrotic cells has the reduced form of Cys106 and can initiate inflammation, whereas HMGB1 released during apoptosis has Cys106 in an oxidized state that is unable to stimulate TLR4; as a result, the inflammatory response is not induced.[14,38] In addition to the importance of a thiol group at Cys106, TLR4 stimulation by HMGB1 requires a concomitant disulfide Cys23–Cys45 linkage.[40]

The intrinsic immune activity of HMGB1 has been a subject of controversy, although this situation might reflect the protein preparation used and the redox state of the cysteine residues. Indeed, commercially available recombinant HMGB1 preparations can contain the reducing agent dithiothreitol which disrupts the crucial disulfide bond.[40] These preparations are unable to stimulate cytokine production and their use in experiments has led to conflicting reports regarding the capacity of HMGB1 to induce inflammation. Although HMGB1 can induce

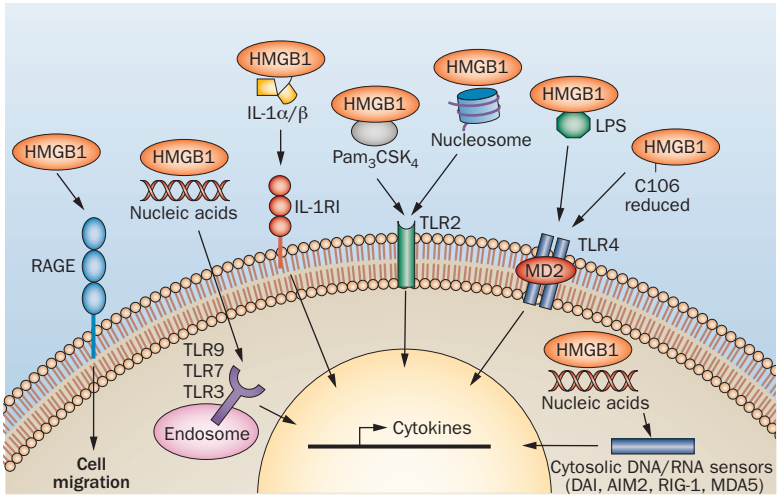


Figure 3 | Signaling by HMGB1. Extracellular HMGB1 elicits responses by activating multiple signaling pathways. HMGB1 induces cytokine production, migration, proliferation and differentiation by interaction with TLR2, TLR4, TLR9 and RAGE. The induction of cytokine production requires activation of TLR4 by HMGB1 with a disulfide bond between Cys23 and Cys45 and a reduced Cys106. The influence of redox modifications on HMGB1 interactions with other receptors is presently unknown. In addition to direct receptor interactions, HMGB1 can form complexes with selected ligands and thereby enhance the responses induced by the bound ligand. The molecular pathway underlying this synergy is not known although dependence on signaling through the ligand receptor and independence of TLR2, TLR4 or RAGE as HMGB1-binding coreceptors has been demonstrated. The formation of a ternary complex with CD24, Siglec-10 and HMGB1 activates the inhibitory receptor Siglec-10 and thereby can dampen signaling from activating receptors. To date, this pathway is the only down-regulatory effect involving HMGB1 signaling. Abbreviations: LPS, lipopolysaccharide; RAGE, receptor for advanced glycation end products.

inflammation, it can also mediate anti-inflammatory effects by inhibiting NFκB nuclear translocation induced by the interaction of HMGB1 and TLR4.[35]

Independent of its redox state and intrinsic cytokine-inducing ability, HMGB1 can promote inflammation by forming immunostimulatory complexes (with mediators including IL-1, endotoxin and additional partner molecules such as DNA) with unique molecular properties.[41–44] In the context of innate immunity in the extracellular space, complexes can enhance responses dramatically when compared with induction by the ligand alone. Indeed, evidence has suggested that HMGB1–partner molecule complexes signal through the receptor of the partner molecule, although through an as yet unknown mechanism.[44] These complexes have been identified primarily by immunoprecipitation and costimulation assays and, at present, only limited information is known about the nature of the structures formed and the various HMGB1 residues mediating the interaction with other molecules.[45] Furthermore, the kinetics of complex formation have not been well defined, although studies suggest that the *in vitro* interaction of HMGB1 and lipopolysaccharide is time and concentration dependent and can occur over many hours.[44] These findings could suggest that conformational changes in the partner molecules are required for a stable interaction.

Although the function of cytoplasmic HMGB1 is not known, it might act as a 'universal' sensor of cytosolic nucleic acids. Indeed, absence of cytosolic HMGB1 proteins severely impairs induction of type I interferon and other immune responses by DNA or RNA that are targeted to activate cytosolic and endosome-based nucleic-acid-sensing receptors.[46,47] Figure 3 illustrates the diverse mechanisms by which HMGB1 can signal cells as an alarmin, alone or in complex with cytokines as well as other endogenous and exogenous molecules.

Autoimmune and inflammatory disease

Arthritis

As an alarmin, HMGB1 has the capacity to drive pathogenesis in highly diverse immune-mediated conditions. Of these conditions, rheumatoid arthritis (RA) was one of the first settings in which this role was demonstrated convincingly. A role for HMGB1 in the pathogenesis of RA (the most common form of inflammatory arthritis) is supported by studies showing an increase in extracellular HMGB1 expression in blood, synovial tissue and synovial fluid;[48–51] joints from rodent models of RA show similar findings (Figure 4).[52,53] Further evidence of the role of HMGB1 in joint inflammation comes from studies indicating disease attenuation by agents (including polyclonal and monoclonal anti-HMGB1 antibodies, recombinant A box domain of HMGB1, recombinant thrombomodulin, soluble RAGE, gold salts and corticosteroids) which antagonize the expression or the activity of HMGB1.[49,54–60] Importantly, neutralization of HMGB1 in experimental animal models can protect against the characteristic cartilage and bone destruction of RA.

Studies of RA synovial biopsies from patients with RA indicate aberrant HMGB1 expression in the pannus tissue at the cartilage–bone interface as well as in areas with tissue hypoxia.[58] The release of HMGB1 from stressed or dying hypoxic cells might boost the production of other proinflammatory molecules including TNF and IL-1,[15] suggesting that HMGB1 couples hypoxia and inflammation in arthritis. Indeed, data from *in vitro* models indicate that activated synovial macrophages, fibroblasts and vascular endothelial cells both respond to and secrete HMGB1 in synovitis, suggesting diverse sources of HMGB1 production and target activity. Consistent with the capacity of HMGB1 for immune activation, single HMGB1 injections into rodent joints can induce long-lasting, destructive arthritis.[53]

HMGB1 can form complexes with IL-1α and IL-1β to enhance immune responses.[41,61–63] These complexes might be particularly relevant for joint inflammation—where all three factors are present. The importance of this pathway is supported by observations that single intra-articular HMGB1 injections in mice deficient for the receptor of IL-1 fail to generate synovitis.[61] As HMGB1 can bind other partner molecules, such as bacterial DNA, viral RNA, or endotoxin, the resulting complexes might also induce proinflammatory cytokine production.

Systemic lupus erythematosus

In addition to its activity in RA, HMGB1 might also promote the pathogenesis of SLE—a prototypic autoimmune disease characterized by multisystem involvement in

association with antibodies to components of the cell nucleus (termed antinuclear antibodies). These antinuclear antibodies bind diverse nuclear macromolecules and can mediate disease by forming immune complexes that promote cytokine production and can deposit in tissue. In SLE, the enhanced expression of nuclear molecules (including HMGB1), which could result from either increased levels of cell death or impaired clearance of dead cells,[64] could lead to inflammation either locally or systemically. Thus, in skin lesions in patients with SLE, HMGB1 shows increased expression in the epidermis as well as dermal infiltrates in association with IL-1β and TNF; in these lesions, HMGB1 is present in the cytoplasm of cells as well as in the extracellular space.[65] Although the cells releasing HMGB1 have not been definitively identified in this setting, extracellular HMGB1 could result from either activation or the death of keratinocytes or infiltrating immune cells.[65] Importantly, ultraviolet radiation can enhance the translocation of HMGB1 to the cytoplasm and extracellular space in the skin of patients with cutaneous lupus, suggesting a mechanism for photosensitivity that flares disease.[66]

In addition to local inflammation, HMGB1 could promote pathogenesis by disturbing the cytokine milieu. As shown by Western blotting, levels of HMGB1 are increased in the blood of patients with SLE and can correlate with disease activity.[67–69] At least some extracellular HMGB1 exists in the form of immune complexes comprising anti-DNA autoantibodies and DNA.[70] These complexes can induce type I interferon production by plasmacytoid dendritic cells by stimulating TLRs, non-TLR nucleic acid sensors, and other receptors such as the Fc receptor.[71–73]

In the original studies of cytokine-inducing complexes, addition of purified DNA to anti-DNA autoantibodies created an active moiety.[71] This finding suggested that immunostimulation requires only a source of DNA to generate pathogenic complexes from autoantibodies. Subsequent studies, however, demonstrated that circulating immune complexes from patients with SLE contain HMGB1, which is essential for activating the immune response.[70] Other studies showed that HMGB1 can interact with DNA to enhance stimulation of plasmacytoid dendritic cells by a mechanism that involves both RAGE and TLR9.[70]

The importance of HMGB1–DNA complexes in SLE was demonstrated further in studies showing that immunization with HMGB1–DNA complexes can induce anti-DNA antibody production in mice under conditions in which DNA alone is inactive.[42] In these immunization experiments, antibody production was dependent on the presence of TLR2, one of the receptors with which HMGB1 can interact.[42] These findings provide evidence for the plasticity in receptor interactions of HMGB1 complexes and the diversity of potential interacting partners. Furthermore, cells undergoing apoptosis can provide a source of DNA–HMGB1 complexes.[42] These observations are notable in view of studies indicating that, during apoptosis, HMGB1 becomes more tightly adherent to chromatin following post-translational modification.[10] Although release of DNA–HMGB1 during apoptosis is consistent with the dynamics of nuclear molecular translocation during this form of death, apoptosis has often been viewed as anti-inflammatory in nature. Nevertheless, when DNA–HMGB1 complexes are separated from cells, they might have more proinflammatory activity.

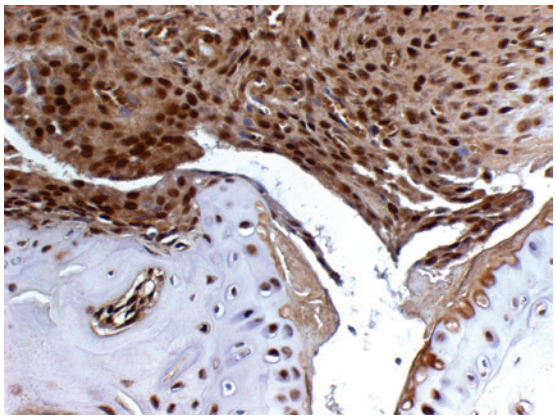


Figure 4 | HMGB1 expression in mouse collagen-induced arthritis. The photo illustrates HMGB1 expression in the synovium from a mouse with collagen-induced arthritis. The micrograph was obtained from a paraffin-embedded articular section displaying immunohistochemical diaminobenzidine staining (brown) of HMGB1 in the synovitis including pannus tissue penetrating the articular cartilage. Synovium contains cells with aberrant HMGB1 expression. Thus, in addition to the expected nuclear location, HMGB1 shows cytoplasmic and extracellular expression.

Although the presence of HMGB1 in immune complexes could result from DNA attachment, patients with SLE or other autoimmune diseases can express antibodies to HMGB1 itself.[26,27,74,75] These antibodies can bind to a variety of epitopes along the HMGB1 molecule; although the antibodies are not specific to SLE, their levels might correlate with disease activity.

Myositis

Another autoimmune disease where HMGB1 might drive pathogenesis is myositis or inflammatory myopathy. As shown by histopathology, HMGB1 expression is increased during myositis and has cytoplasmic and extracellular expression in the invading inflammatory infiltrates and endothelial cells; control samples from healthy donors have no cytoplasmic HMGB1 expression. Importantly, a comparison of biopsies taken prior to and after prednisone therapy showed a reduction in extra-nuclear expression of HMGB1. This reduction is due mainly to a decrease in inflammatory cell infiltrates whereas the staining intensity in muscle cells and endothelial cells remains similar.[76]

A direct role for HMGB1 as an instigator of myositis is suggested by the colocalization of cytoplasmic HMGB1 expression with upregulated MHC class I expression on muscle fiber specimens obtained early in disease. The relevance of this finding is substantiated by studies indicating that stimulation of healthy mouse skeletal muscle fibers with IFN-γ can induce cytoplasmic

HMGB1 expression; furthermore, *in vitro* stimulation with extracellular HMGB1 can cause an upregulation of MHC class I molecule expression, with extracellular HMGB1 leading to decreased calcium release from the muscle fibers and irreversible muscle fatigue during tetanic stimulation.[77]

Although this study supports a role of HMGB1 in inflammation during myositis,[77] other studies have demonstrated that HMGB1 is expressed in the cytoplasm of regenerating muscle fibers recovering from ischemia and that HMGB1 can act as an angiogenic and myogenic inducer if injected intramuscularly.[78–80] Thus, it is possible that HMGB1 has a dual role in myositis: in early disease HMGB1 can drive inflammation, upregulation of MHC class I expression and muscle fatigue, as disease progresses, it could also promote tissue protection and regeneration.

Other inflammatory diseases

Analysis of either blood or biopsy samples from patients has provided compelling evidence that HMGB1 contributes to the immunopathogenesis of diverse rheumatological conditions. Thus, in the minor salivary glands of patients with Sjögren's syndrome, HMGB1 shows increased expression in the infiltrating mononuclear cells in association with increased expression of IL-1β and TNF.[81] Similarly, a role of HMGB1 in vasculitic diseases of adults and children is suggested by evidence of increased levels in the blood.[82–88] In vasculitic diseases, levels of HMGB1 might serve as a marker of disease activity as well as a predictor of response to treatment. The list of diseases in which HMGB1 has been implicated is likely to grow as more conditions are investigated, especially with improved assay techniques.[1]

Among non-rheumatic conditions in which HMGB1 might be pathogenic, the study of acetaminophen-induced liver toxicity could provide insights that are relevant to rheumatic diseases. Acetaminophen is a widely used analgesic that remains a major cause of drug-induced liver injury. Acetaminophen overdosing can result in toxic levels of the metabolite NAPQI, which leads to hepatocyte damage and death, and, ultimately, to irreversible liver failure.[28]

In an elegant study to clarify these mechanisms in a mouse model of acetaminophen-induced liver toxicity, Antoine *et al*.[38] observed that animals fed prior to acetaminophen administration had better survival than mice starved for 24 h prior to acetaminophen challenge. Nevertheless, the sera from both treatment groups showed similarly increased levels of HMGB1. Analysis of the mode of hepatocyte cell death by histopathology indicated that loss of hepatocytes in fed animals was mainly through apoptosis, although necrosis also occurred. By contrast, in fasted mice, the dominant mode of hepatocyte cell death was necrosis. Importantly, the extent of liver inflammation correlated well with the degree of necrotic cell death.[38]

An analysis of the redox status of HMGB1 in serum provided a valuable clue to the basis of these differences. Liquid chromatography-tandem mass spectrometry analysis demonstrated that HMGB1 Cys106 in fed mice was mainly oxidized whereas HMGB1 in starved mice contained mainly the reduced form of this cysteine residue. As HMGB1 blockade can increase the survival rate in the fasted group of mice (where necrosis predominates), these studies indicate that the redox state of released HMGB1 influences its biological activity and depends on mechanisms for its release (that is, necrosis and immune activation versus apoptosis).[38,40]

Blockade of HMGB1 as therapy

At present, clinical studies using HMGB1-specific antagonists have not been performed in patients, although this approach is effective in preclinical animal models of diverse conditions including sepsis, arthritis, stroke, organ transplantation and acetaminophen-induced hepatotoxicity (Supplementary Table 2).[1] Therapy based on the use of the recombinant A box domain of HMGB1 has also been successful, although the mechanism is unknown.[1] Of note, small molecules can also antagonize HMGB1 by blocking its release from cells, with metformin, for example, inhibiting the release of HMGB1 from a macrophage cell line as well as increasing survival in mice treated with lipopolysaccharide.[89]

The setting of sepsis is especially informative and suggests unique effects of inhibiting HMGB1. Administration of anti-HMGB1 antibodies or recombinant A box protein in mice with polymicrobial gram-negative sepsis from cecal ligation and perforation substantially improves survival even when treatment is started late in disease. By contrast, anti-TNF antibodies worsen survival in this model, highlighting important differences between the roles of HMGB1 and TNF in pathogenesis.[90] Whether HMGB1 blockade in arthritis would provide any advantage when compared with TNF neutralization is presently unknown as the models studied are dependent on TNF as well as HMGB1.

Conclusions

HMGB1 is a prototype alarmin that can induce immune responses by itself as well as in association with other endogenous and exogenous molecules. Clinical and experimental studies suggest that HMGB1-dependent inflammatory mechanisms have a key role in the pathogenesis of autoimmune and inflammatory diseases, with HMGB1 potentially an important new biomarker. Consistent with a role of HMGB1 as a disease mediator, agents targeting this molecule have shown success in animal models; these agents include neutralizing anti-HMGB1 monoclonal antibodies, recombinant A box domain of HMGB1 and compounds that cause intracellular HMGB1 retention. In developing HMGB1 antagonists for clinical use, safety will be an important issue, with concerns related to the complexity of HMGB1 biology and the pleiotropic and even competing functions of extracellular HMGB1 as it interacts with multiple signaling systems. Furthermore, dynamic post-translational modifications of HMGB1 might shift its activity from inflammation to tissue repair. In the future, studies will further define the immunological properties

of HMGB1 and optimize strategies for blocking its activity in autoimmune and inflammatory disease. As the amino acid sequence of HMGB1 is highly conserved in all mammals, rapid translocation of antagonists developed in animals to the setting of human disease should be possible and hopefully new approaches to therapy will be opened.

Review criteria

We searched PubMed with terms "HMGB1", "alarmin", "DAMP", "rheumatoid arthritis", "systemic lupus erythematosus", "myositis", "Sjogren's syndrome", "autoimmunity", "acetaminophen", "endotoxin" and "autoantibodies". We did not restrict the years of this search.

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Author contributions

All authors contributed equally to all aspects of the article.