The role of high mobility group box chromosomal protein 1 in rheumatoid arthritis

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Abstract

High mobility group box chromosomal protein 1 (HMGB1) is a ubiquitous highly conserved single polypeptide in all mammal eukaryotic cells. HMGB1 exists mainly within the nucleus and acts as a DNA chaperone. When passively released from necrotic cells or actively secreted into the extracellular milieu in response to appropriate signal stimulation, HMGB1 binds to related cell signal transduction receptors, such as RAGE, TLR2, TLR4 and TLR9, and becomes a proinflammatory cytokine that participates in the development and progression of many diseases, such as arthritis, acute lung injury, graft rejection immune response, ischaemia reperfusion injury and autoimmune liver damage. Only a small amount of HMGB1 release occurs during apoptosis, which undergoes oxidative modification on Cys106 and delivers tolerogenic signals to suppress immune activity. This review focuses on the important role of HMGB1 in the pathogenesis of RA, mainly manifested as the aberrant expression of HMGB1 in the serum, SF and synovial tissues; overexpression of signal transduction receptors; abnormal regulation of osteoclastogenesis and bone remodelling leading to the destruction of cartilage and bones. Intervention with HMGB1 may ameliorate the pathogenic conditions and attenuate disease progression of RA. Therefore administration of an HMGB1 inhibitor may represent a promising clinical approach for the treatment of RA.

Key words: HMGB1, rheumatoid arthritis, signal transduction, HMGB1 inhibitors.

Introduction

High mobility group box chromosomal protein 1 (HMGB1) is a histone chromosome-binding protein widespread in the mammal eukaryotic cell nucleus. HMGB1 is a highly conserved single polypeptide of 25 kDa containing two DNA-binding domains (HMG box A and B) and a negatively charged C terminus [1–3]. Intranuclear HMGB1 is known to act as a nuclear DNA chaperone that participates in DNA replication, transcription, recombination, and repair, as well as in nucleosome formation and in regulation of the transcriptional activity of steroid hormone receptors and glucocorticoid receptors [4–7]. HMGB1 knockout mice die of hypoglycaemia within 12 h after birth due to insufficient glucocorticoid receptor expression, while HMGB1−/− cell lines can grow normally in vitro, which suggests that HMGB1 is not necessary for the organization of nuclear chromatin but is essential for proper transcriptional control [8]. Both box A and box B can combine with DNA and are involved in DNA double chain folding and distortion [5]. Box B is the protein transduction domain, and purified box A can act as an antagonist of HMGB1, although its half-life is short. In addition, the C-terminal acidic tail was suggested to be responsible for the inhibitory effects of HMGB1 on efferocytosis [9].

HMGB1 was recognized as a potential late inflammatory cytokine in Wang’s study regarding the progress of sepsis and endotoxaemia in 1999 [10]. Therefore, it was gradually found that extracellular HMGB1 protein, an endogenous damage-associated molecular pattern (DAMP), acts as a proinflammatory mediator involved in the pathogenesis of many inflammatory diseases such as arthritis, acute lung injury, graft rejection immune response, ischaemia reperfusion injury and autoimmune liver damage [11–15].
In addition, HMGB1 enhances eosinophil survival and acts as a chemoattractant and a mitogen, as well as reducing skeletal muscle regeneration and remodelling [16, 17]. As a danger signal, HMGB1 plays a much more important role in the progress of disease development and aggravation than originally thought.

In addition, HMGB1 has been shown to modulate phagocytosis of apoptotic neutrophils, which suggested an alternative mechanism to amplify the inflammatory response [18]. Furthermore, endogenous HMGB1 was recognized as a redox-sensitive regulator of autophagy. Reducible HMGB1 binds to receptor for advanced glycation end products (RAGE), induces beclin-1-dependent autophagy and promotes tumour resistance, while oxidized HMGB1 induces apoptosis mediated by the intrinsic caspase-9/-3 pathway [19–22]. Mutation of cysteine 106 or formation of an intramolecular disulphide bridge between Cys23 and Cys45 binding to beclin-1 resulted in sustained autophagy, while HMGB1 inhibition or loss limited oxidative stress-induced autophagy [23, 24].

**Origin of extracellular HMGB1**

Nuclear HMGB1 fulfils a proinflammatory role in the extracellular matrix. HMGB1 is actively secreted by monocytes, macrophages, NK cells, dendritic cells, endothelial cells, synovial fibroblasts, platelets, astrocytes and tumour cells in response to stimulation by endogenous cytokines, Toll-like receptor (TLR) ligands and some common non-specific highly conserved molecular structures in pathogenic microorganisms, such as lysophosphatidylcholine (LPS) [25, 26]. Released HMGB1 accompanied by IL-1β or nucleosomes could augment inflammatory effects [27]. In addition, HMGB1 can be passively released during cell necrosis or damage. HMGB1—necrotic cells showed a minimal inflammatory response in vitro, which may be explained by the release of HMGB1 serving as a death signal to neighbouring cells [28].

During apoptosis, generalized underacytlation of histones enhance the affinity of nuclear HMGB1 and chromatin, leading to no or minimal HMGB1 release [28]. Most recent studies have indicated that apoptotic cells can also release HMGB1, which undergoes oxidative modification on Cys106 and delivers tolerogenic signals to suppress immune activity rather than acting as a proinflammatory signal [29]. In a mouse model deficient in macrophage function to digest DNA in apoptotic cells, HMGB1 was released and induced the development of chronic polyarthritis similar to RA [30]. If professional phagocytes have impaired ability to remove apoptotic cells and undigested apoptotic bodies, cells undergo secondary necrosis leading to late HMGB1 release and production of inflammatory mediators [31].

In all nucleated cells, HMGB1 shuttles actively between the nucleus and cytoplasm [32]. Upon activation of LPS, HMGB1 is extensively acetylated and phosphorylated and is localized in the cytosol [32]. Unlike other secreted proteins, which are folded and modified by the rough endoplasmic reticulum and Golgi body and form a protuberance at the edge of the Golgi body for transport, HMGB1 has been shown to be secreted by monocytes in a non-classical vesicle compartment-mediated pathway involved in exocytosis of secretory lysosomes into the extracellular matrix, together with the secretion of early inflammatory mediators such as IL-1β. It is worth noting that IL-1β is released in an autocrine manner earlier after ATP activation, while HMGB1 is generated later by triggering LPS in inflammatory sites [33]. Moreover, tissue hypoxia or ischaemia will also induce active HMGB1 secretion and accelerate the development of arthritis, although the detailed mechanism underlying this process is not yet clear [24].

**Mechanism of HMGB1 expression/ regulation**

Extracellular HMGB1 participates in the pathogenesis of initiation and promotion of inherent immunity through binding to various receptors on the surface of responding cells. The signal transduction of extracellular HMGB1 is closely associated with RAGE, TLR2, TLR4, TLR9 and CD24-Siglec-10 (Siglec-G in mice) [34–37]. HMGB1 combined with these receptors leads to activation of the NF-κB pathway, inducing transcription of multiple proinflammatory genes and upregulating leucocyte adherence molecules, thus initiating and maintaining an inflammatory reaction cascade [38]. In particular, receptors TLR2, TLR4, IL-1RI and TLR9 activated by HMGB1 form complexes with ssDNA, LPS, IL-1β and nucleosomes, respectively, rather than by the HMGB1 molecule itself [27]. The inter-action and downstream signals can be altered by post-translational modification of HMGB1 on its cysteine residues, such as acetylation, phosphorylation, methylation and redox [39]. The first demonstrated receptor was RAGE, a transmembrane protein of the immunoglobulin superfamily of cell surface molecules. RAGE regulates HMGB1 expression through transient phosphorylated mitogen-activated protein kinases (such as JAK/STAT, ERK1/2, p38-MAPK and p42/44 kinase) to activate the NF-κB pathway and induce inflammation [40, 41]. TLR4 is the dominant receptor for HMGB1 to induce cytokine release. Through interaction with TLR4, HMGB1 can activate the inhibitor of kinases IkKα and Ikκβ to phosphorylate NF-κB, which is slightly different from RAGE by activating Ikκβ [11, 42]. The co-localization of HMGB1 and TLR4 leads to intramolecular disulphide bond formation between Cys23 and Cys45 and reduction of Cys106, which are critical for the nucleocytoplasmic shuttle [42, 43]. TLR2 and TLR9 are common receptors for HMGB1, and CD14 may be a universal adaptor transmission signal from DAMP/PAMP in the presence or absence of TLR2 to induce necrotic cells to activate NF-κB, while MD2 recognizes only exogenous PAMP in the presence of TLR2-CD14 [44]. In addition, HMGB1 can bind to IL-1β, CXCL12, syndecan, triggering receptors expressed on myeloid cells 1 (TREM1), macrophage adherence molecule 1 (MAC1) or synergy with bacterial DNA, viral RNA and endotoxin to initiate chronic inflammatory effects [45–47].

In contrast, HMGB1 associated with Siglec-10 (human)/G (mouse) through CD24 can repress HMGB1 activation.
by allowing Siglec10/G-associated phosphatases, such as SHP1, to repress the initiated inhibitory receptor Siglec10 signal, and thus play a negative regulatory role in NF-κB activation [37, 48]. Hsp72 is a negative regulatory factor in the regulation of HMGB1. Overexpression of heat shock protein 72 (HSP72) prevents HMGB1 cytoplasmic translocation and release after LPS, TNF-α or oxidative stress treatment, strongly inhibiting HMGB1-induced cytokine expression and release, which is closely correlated with inhibition of the MAPKs and the NF-κB pathway [49]. In addition, binding of HMGB1 to thrombospondin, a glycoprotein predominantly expressed on the surface of vascular endothelial cells and that mediates thrombin-dependent activation of protein C, may also provide important negative signals to modulate coagulation [46] (Fig. 1).

The regulatory mechanism of HMGB1 secretion is mainly based on a positive feedback system. In vitro, TNF-α stimulates HMGB1 phosphorylation and release from monocytes, mononuclear macrophages and endothelial cells. Conversely, existing HMGB1 can activate NF-κB, leading to TNF-α, IL-1β and IL-6 release, thus forming an inflammatory reaction positive feedback [38, 50]. Notably, cytokines released by HMGB1 stimulation were biphasic and delayed, with the release peaks at the 3rd hour and 8th to 10th hour, respectively [51].

HMGB1 also participates in the regulation of adaptive immune responses. The autocrine/paracrine-released HMGB1 could improve the antigen-presenting ability of dendritic cells and macrophages through upregulation of MHC II, CD83, CD80 and CD86 expression on the surface of macrophages, induction of dendritic cell maturation and migration, activation of T helper cells and B lymphocytes and promotion of CD4+ T cell proliferation [52–54].

HMGB1 in RA

RA is an autoimmune disease characterized by chronic synovial inflammation and erosion of the articular cartilage and bone. The prevalence of the disease is 0.51% worldwide. With the progression of RA, vasculitis lesions involve organs throughout the whole body. The exact causes of RA are not fully understood, while the overproduction of proinflammatory cytokines (such as TNF-α, IL-1 and IL-6) and metalloproteinases by IA synoviocytes, macrophages and monocytes plays a critical role in the development and exacerbation of the disease [55].

HMGB1 has been shown to be important in the pathogenesis of rheumatoid diseases, especially arthritis, RA, OA, JIA, SLE, SS, PM, DM and AS [56–61]. Serum levels of HMGB1 and HMGB1 nucleosome complex in most of these diseases are significantly higher than in healthy controls and are correlated to an extent with disease activity. Although serum HMGB1 levels in patients with primary knee OA were very low and did not show a significant difference compared with healthy controls, HMGB1 levels in SF of knee OA patients were significantly higher than in healthy controls and showed a positive correlation with radiographic disease severity [56]. In LN patients, increased urinary HMGB1 levels are especially closely correlated with SLEDAI, and their renal tissue also shows increased HMGB1, together with increased expression of TLR2, TLR4 and RAGE [62]. Immunohistochemical staining showed elevated levels of extracellular HMGB1 in biopsy samples from the minor labial salivary glands of patients with SS [59]. Cytoplasmic and extracellular HMGB1 expression were detected in infiltrating rounded mononuclear cells, vascular endothelial cells and muscle fibres of PM and DM patients, and the aberrant expression of HMGB1 could be downregulated by high-dose corticosteroid treatment [60]. A most recent report postulated that the serum level of HMGB1 was significantly higher in AS than healthy controls, although the association with disease activity and quality of life seems obscure [61]. In particular, there has been a great deal of research regarding the association between HMGB1 and RA from the viewpoint that HMGB1 acts as an important proinflammatory cytokine participant in the development and progression of RA disease (Fig. 2).

Aberrant expression of HMGB1

Kokkola et al. [63] first proposed the abundant nuclear, cytoplasmic and extracellular expression of HMGB1 in the synovial tissues of RA patients and rats with experimental arthritis. Taniguchi et al. [64] further demonstrated that HMGB1 plays a pivotal role in the pathogenesis of RA. In their studies, they showed that HMGB1 level was higher in serum, SF and synovial tissue of RA patients than in those of OA patients and healthy human controls. The synovial fluid macrophages (SFM)s enhanced the expression of RAGE and released TNF-α, IL-1β and IL-6 upon HMGB1 stimulation. In turn, under stimulation with TNF-α, HMGB1 was not only upregulated at the mRNA level but also translocated from the nucleus to the cytoplasm. The serum level of HMGB1 in RA patients is associated with DAS [65]. Shortly afterwards, Pullerits et al. reported that injection of recombinant HMGB1 (rHMGB1) into mice successfully induced arthritis characterized by mild to moderate synovitis and pannus formation [66]. Using immunohistochemical staining, aberrant HMGB1 deposition was visible in cytoplasmic and extracellular inflammatory synovial tissue preceding the onset of clinical signs of experimental CIA and became remarkable after the onset of disease [67].

Recently Ostberg et al. [68] utilized DNase II(−/−) x IFNRI(−/−) mice, which spontaneously develop symmetric polyarthritis and are a good model of RA, and further theorized the involvement of HMGB1 in the pathogenesis of RA as well as the beneficial effects of antagonistic treatment targeting HMGB1. In addition, HMGB1 can promote the activation and polarization of Th17 cells through upregulation of TLR2 and Th17 cell-associated cytokines from RA patients, and the increased Th17 cells may partly contribute to the immune dysregulation in RA [69, 70].

Receptor of HMGB1 and signal transduction

HMGB1 is expressed predominantly in the cytoplasm and extracellular matrix of endothelial cells, macrophages, fibroblast-like synoviocytes and synoviocytes in RA.
Macrophages in the synovium of RA patients show high levels of RAGE, TLR2, TLR4 and IL-1RAcP complex expression, and stimulation by rHMGB1 enhanced the release of inflammatory factors such as TNF, IL-1β and IL-6. The HMGB1/RAGE system is the major signal transduction system involved in the production of inflammatory mediators, and blockade by the soluble form of RAGE (sRAGE) significantly inhibited the release of TNF-α from cultured SFMs [64]. TLR2 and TLR4 ligation were observed with activation of RA SFMs, thus supporting the potential roles of TLR2 and TLR4 in the inflammation and joint destruction associated with RA [71]. IL-1RAcP complex in HMGB1-triggered joint inflammation partly mediated by the NF-κB pathway and IL-1RAcP complex-deficient mice did not develop arthritis after microinjection of rHMGB1 into the joints [66]. Intracellular HMGB1 in macrophages/monocytes transactivates IL-1β promoter through physical interaction with PU.1 (a myeloid and B cell-specific transcription factor belonging to the Ets family), resulting in maintenance and amplification of inflammatory effects [72]. HMGB1 has been reported to strongly enhance the production of proinflammatory cytokines in a synergistic manner with TLR2, TLR4 and TLR9 ligands, while the equivalent amount of HMGB1 alone showed no such effect [73]. Moreover, Wåhåmaa et al. [74] demonstrated that HMGB1 in complex with LPS, IL-1β or IL-1α increased the production of inflammatory factors, such as TNF, IL-6, IL-8 and MMPs from cultured synovial fibroblasts of RA, and the synergistic effects were mediated through the partner ligand-reciprocal receptors TLR4 or IL-1RAcP complex.

In addition, RA patients show significantly decreased serum and SF levels of sRAGE, the soluble form of RAGE, which has the same ligand-binding specificity as RAGE, equivalent to the extracellular domain of RAGE lacking the cytosolic and transmembrane domains and thus blocking proinflammatory ligands from reaching membrane RAGE [75]. The lack of interaction between HMGB1 and RAGE blockade by sRAGE may be partially responsible for RA.

Extracellular HMGB1 is an osteoclast activator
Osteoclasts are major factors in the destruction of cartilage and support structures in the joints of RA patients. HMGB1 is a strong activator of osteoclasts, which are responsible for the destruction of bone and cartilage in RA. HMGB1 interacts with toll-like receptors (TLRs) and triggers the release of inflammatory mediators such as TNF-α, IL-1β, and IL-6, leading to the activation of osteoclasts. This process is crucial in the progression of joint destruction in RA.

Extracellular HMGB1 is both passively released from necrosis cells and actively secreted by monocytes, macrophages, NK cells, dendritic cells, endothelial cells, synovial fibroblasts, platelets, astrocytes and tumour cells. HMGB1 interacts with RAGE or CD24-Siglec-10/G or forms complexes with ssDNA, LPS, IL-1β and nucleosomes by interaction with TLR2, TLR4, IL-1RI and TLR9, respectively. HMGB1 also binds to CXCL12, TREM1 or MAC1, which could augment inflammation effects. Only a small amount of HMGB1 release occurs during apoptosis, which undergoes oxidative modification on Cys106 and delivers tolerogenic signals to suppress immune activity.

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can be released from osteoclasts and osteoblasts, and the latter release HMGB1 in response to PTH, which could mediate bone remodelling [76]. During the pathological process of endochondral ossification, HMGB1 shows a chemotactic effect on osteoclasts and osteoblasts [77]. Intriguingly, within the bone marrow, HMGB1 can also be released from apoptotic bone cells, as a bone resorption signal induces inflammatory bone loss via inflammatory cytokine release by living stromal cells, while the apoptotic HMGB1 release decreases as osteoblasts mature [78]. It has been reported that HMGB1 can accelerate t-PA-catalysed plasminogen and metalloproteinase activities as well as its own degradation, and a process has been proposed by which it may contribute to the cartilage and bone destruction seen in joints in the early stages of arthritis [79]. HMGB1 and TNF-α enhanced the role of receptor activator for NF-κB ligand (RANKL)-induced and integrin-dependent osteoclastogenesis by interaction with RAGE both in vitro and in vivo, which indicated that HMGB1 and TNF-α play prominent roles in the abnormal regulation of osteoclastogenesis and bone remodelling [80-82]. Furthermore, using in situ hybridization techniques, HMGB1 mRNA was shown to be highly upregulated in areas of cartilage and bone destruction in CIA synovitis specimens [83]. The expansive synovial tissue called pannus is composed of macrophages, osteoclasts and invasive fibroblast-like synoviocytes [84]. Pannus behaves like an invasive tumour, progressively eroding cartilage, subchondral bone and ligament tendons through isolation of bone nutrition and release of inflammatory mediators and proteolytic enzymes. High levels of HMGB1, TNF-α and IL-1β expression were detected in pannus of arthritis models, which may be induced by ischaemia in arthritis tissue, activated complement and proinflammatory cytokines [46].

Extracellular HMGB1 promotes synovial cell proliferation

The synovium is rich in active and excessive proliferation of immunocompetent cells in the damaged joints of RA. Blood vessel proliferation is common in these invasive joints [85]. The sublining of the synovium shows changes in the cell type ratio, leading to susceptibility to synovial inflammation, while the intimal lining of the synovium shows a remarkable increase in the cell population [84]. Cadherin-11 knockout mice deficient in intimal lining development are resistant to arthritis-induced cartilage damage [85]. A recent study suggested that HMGB1 induces synovial cell proliferation by activating the STAT1 signal pathway [86].

Intervention with HMGB1

Although the clinical effects have not been tested, therapeutic administration of specific neutralizing HMGB1 antibodies or truncated HMGB1 box A protein showed...

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**Fig. 2** Abnormal expression of extracellular HMGB1 in RA.
remarkable effects in ameliorating the pathogenic condition and attenuating disease progression in the CIA mouse model [87–89], which may provide a new target for biologic therapy for RA. Ethyl pyruvate was first described as an effective pharmacological inhibitor of HMGB1 protein synthesis and secretion [90] and has been shown to successfully prevent systemic inflammation during cardiopulmonary bypass surgery in phase II clinical trials [91]. Thrombomodulin (TM), an endothelial anticoagulant co-factor that promotes thrombin-mediated formation of activated protein C (APC), suppresses inflammation by preventing the interaction of HMGB1 with its receptor [92]. Both TM and ethyl pyruvate represent therapeutic measures for blocking HMGB1. Elevated levels of HMGB1 were shown to be significantly correlated with inhibited vagus nerve activity in RA patients, while this cholinergic anti-inflammatory pathway had protective effects for delaying the bone degradation and synovial inflammation in RA patients [65, 93]. Treatment with the selective α7 subunit of nicotinic acetylcholine receptor (nAChR) agonist nicotine can attenuate inflammatory cytokine production and clinical signs of CIA in mice, together with downregulation and inhibition of synovial HMGB1 translocation [94, 95]. Vagus nerve stimulation activates the cholinergic anti-inflammatory pathway to reduce HMGB1 secretion by acting on the α7 subunit of nAChR present on macrophages [47]. Thus pharmacological treatments targeting the α7 subunit of nAChR may be explored for RA patients. In addition, a new finding suggested that box A protein could reduce the secretion of CXCL12, which is a factor required for attraction of myeloid-derived cells by HMGB1. Interaction between HMGB1 and CXCL12 may contribute to the regulation of leucocyte recruitment and motility [96].

Notably, systemic administration or IA glucocorticoid treatment can significantly reduce serum levels of HMGB1 in RA patients, with beneficial clinical effects, but RA patients with systemic TNF-blocking therapy showed no significant alterations in cytoplasmic or extracellular expression of HMGB1 or in HMGB1 mRNA levels in synovial arthroscopy samples [97–99], which may support the previous suggestion that TNF is not an independent signal of HMGB1 activation in RA [100].

**Conclusion**

As a potent late inflammatory cytokine, HMGB1 plays an important role in the development and progression of RA. HMGB1 levels were higher in the serum, SF and synovial tissues of RA patients compared with healthy controls. Other studies have shown that extracellular HMGB1 contributes to the formation of pannus, destruction of cartilage and bones and proliferation of synoviocytes, while the precise pathogenic mechanism has to be studied further. HMGB1 in complex with LPS, IL-1α or IL-1β or in a synergistic manner with TLR2, TLR4 and TLR9 ligands could increase the production of inflammatory cytokines and MMPs. In the serum and SF of RA patients, sRAGE was significantly decreased, which could block the interaction between HMGB1 and RAGE.

So far, scientists have found various kinds of HMGB1 inhibitors, such as ethyl pyruvate, TM, nAChR agonist nicotine, recombinant HMGB1 box A protein and some specific neutralizing HMGB1 antibodies. These inhibitors have been reported to be effective in inhibiting inflammation, which may offer more of a perspective of therapy for RA patients. In experiments in RA animal models, therapeutic administration of specific neutralizing HMGB1 antibodies or recombinant HMGB1 box A protein could predominantly ameliorate disease progression. Therefore, the clinical application of HMGB1 inhibitors in the treatment of RA patients is full of prospects, although further studies are required to improve the details.

**Rheumatology key messages**

- HMGB1 is a pivotal proinflammatory/inflammatory factor that plays a significant role in the pathogenesis of RA.
- The application of HMGB1 inhibitors may provide a new target for biologic therapy for RA patients.

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Role of HMGB1 in RA


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