Involvement of high mobility group box 1 in the auto-inflammatory process in systemic lupus erythematosus
Schaper, Fleur
Chapter 5

Inhibition of High Mobility Group Box 1 as therapeutic option in autoimmune disease; lessons from animal models

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ABSTRACT

Purpose of the review
High Mobility Group Box 1 is a molecule that has gained much of attention in the last couple of years as an important player in innate immune responses and modulating factor in several (auto)-immune diseases. Furthermore, advancements have been made in identifying the diverse functions HMGB1 can play in the body by studying its receptors, pathways and effects. This review will focus on the modulation of HMGB1 in animal models of (auto)-immune diseases.

Recent findings
In different disease models like sepsis, ischemia/reperfusion and arthritis HMGB1 blocking therapies have been tested and the disease course was shown to be ameliorated.

Summary
These findings indicate that HMGB1 is an important mediator in innate immunity, inflammation and sterile injury. Furthermore HMGB1 might be a new therapeutic target in inflammation and auto-immune diseases, which may be translated to the clinic.

Key points
• HMGB1 serum levels are increased in several (auto)immune diseases, and are mostly correlated with disease activity.
• Inhibition of HMGB1 has been investigated in several different animal models, with beneficial effects on survival and the disease course.
• Nuclear retention of HMGB1 inhibits its translocation to the cytoplasm and extracellular release, thereby inhibiting its inflammatory properties.
• Box A is the most used antagonist of HMGB1, which competes with receptors for binding with HMGB1.
INTRODUCTION

HMGB1 (high mobility group box 1) is a non-histone nuclear protein that, depending on its location and posttranslational modifications, has several functions. HMGB1 is ubiquitously expressed in nuclei, where it binds and stabilizes DNA. Mice deficient for HMGB1 die within 24 hours after birth due to severe hypoglycemia, attributed to an impaired glucose metabolism. In contrast fibroblast cell lines, derived from the same HMGB1−/− mice, can proliferate and survive. However, activation of gene expression by the glucocorticoid receptor was diminished indicating that the effects on transcription are cell-type specific. Thus HMGB1 is not essential for cell growth or survival, but plays a role in the accessibility of DNA by transcription factors (1).

HMGB1 can be translocated from the nucleus to the cytoplasm and extracellular space. When released it has been shown to act as a damage-associated molecular pattern (DAMP). Secretion can occur actively from macrophages and other immune cells or passively during apoptosis and necrosis. When present in the extracellular space HMGB1 promotes several responses, for example the release of inflammatory cytokines like TNF-α and Interleukin (IL)-6 (2-5). Other mechanisms of action include cell migration (6), wound healing and neovascularization (7). It has been shown that lower levels of HMGB1 in diabetic skin were correlated with impaired wound healing, while topical application of HMGB1 to the skin of the diabetic mice accelerated wound healing. In the skin of normal mice the application of HMGB1 had no effects, while inhibition of HMGB1 impaired wound healing in normoglycemic mice (8).

Several different receptors have been implicated in HMGB1-mediated functions, including RAGE (receptor for advanced glycation end products) and TLR2 (Toll-like receptor 2), TLR4, and TLR9 (9). Recent studies show that TLR4 is required for HMGB1 induced cytokine release (10-12) while HMGB1 induced cell migration is the result of activation of CXCR4 by HMGB1-CXCL12 complexes (6,10). Moreover, it has been shown that HMGB1 mediated leukocyte migration or proinflammatory cytokine release are mutually exclusive and dependent on the redox state of HMGB1. Reduction of the cysteines on location 23, 45 and 106 turns HMGB1 into a chemoattractant, while a disulfide bond between the cysteines on location 23 and 45 makes it a proinflammatory cytokine (10). HMGB1 can also be terminally oxidized by reactive oxygen species (ROS), which abrogates both the chemoattractant properties as well as the proinflammatory properties. This form of HMGB1 can be released during apoptosis and as its inflammatory properties are abrogated it is thought to play a role in the immunological tolerance of apoptotic cells (10,13).
Independent of its redox state, HMGB1 can form complexes with several immune stimulants like LPS (lipopolysaccharide), CpG, double stranded DNA, nucleosomes and IL-1 (3,4,14-16). These complexes enhance cytokine production in immune cells and fibroblasts when compared to HMGB1 alone and have been shown to signal through the same surface receptors as required for the noncomplexed ligand (14).

In autoimmune diseases like systemic lupus erythematosus (SLE), Sjögren’s syndrome and rheumatoid arthritis (RA) it has been documented that serum levels of HMGB1 are increased in patients compared to healthy controls (17-19). In patients with RA it has been shown that extracellular HMGB1 is present in synovial tissue. After intra-articular injections with glucocorticoids, the levels of extracellular HMGB1 decreased and beneficial clinical effects were observed (20). Another study obtained synovial fibroblasts from RA patients and stimulated these with HMGB1 alone or in complex with IL-1α or IL-1β. HMGB1 in complex with IL-1 increased IL-6, IL-8, TNFα and matrix metalloproteinase 3 production compared to HMGB1 alone. The presence of HMGB1 together with IL-1α or IL-1β in the inflamed joints in RA could contribute to tissue destruction and enhance joint inflammation (15). In SLE it was shown that patients with active lupus nephritis had higher levels of HMGB1 in serum and urine compared to healthy controls which correlated positively with disease activity (18,21). Furthermore, anti-HMGB1 antibodies, HMGB1 complexed with nucleosomes or DNA were found in the serum of SLE patients (18,22,23). Injection of these complexes in non-autoimmune mice could induce anti-DNA antibody production whereas DNA alone was inactive (23). These findings indicate that HMGB1 might be an important factor in the pathogenesis of SLE.

Given the detrimental proinflammatory effects of HMGB1 a number of recent studies have investigated whether intervention in HMGB1 mediated effects is of benefit in dampening inflammation. In this review we will discuss the deleterious effects of HMGB1 in the context of autoimmune diseases and the relevance of inhibition of HMGB1 as it has been reported in several animal models of autoimmune and inflammatory diseases.

**HMGB1 in arthritis models**

Studies have shown elevated HMGB1 levels in both serum and synovial fluid of RA patients (19). Gold salts were one of the first widely used therapies for RA; however toxicity has limited its use which has declined with the introduction of drugs like methotrexate and biologicals. Gold salts were shown to inhibit HMGB1 translocation from the nucleus to the cytoplasm in macrophages (24). Gold nanoparticles, which are thought to be non-toxic, seem to use the same mechanism as gold salts (25). This mode of action was verified in vitro using activated monocytes. Such studies also showed that besides gold salts, dexamethasone and chloroquine can also...
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inhibit HMGB1 release from monocytes (26). Another agent which has shown beneficial effects on RA and causes nuclear retention of HMGB1 is oxaliplatin (27). This agent is also in use as an anti-cancer drug which acts through inhibiting cell division by creating inter- and intra-strand cross links in DNA. Thus, several existing therapies seem to also have an effect via HMGB1 retention.

The clinical effects of neutralizing anti-HMGB1 antibodies have been investigated in rodent models of collagen-induced arthritis (CIA). In these studies both rats and mice were injected twice daily with neutralizing HMGB1 antibodies or the Box A antagonist. Box A is an antagonist for HMGB1 binding to its receptor RAGE, and inhibits HMGB1 effects. Beneficial effects were observed as both mice and rats showed decreased mean arthritis scores, less weight loss and less articular cartilage destruction in comparison to vehicle treated animals (28,29). Another model employed mice deficient for both DNase II and Interferon type 1 receptor

![Figure 1: Schematic presentation of release and signaling of HMGB1 including ways to intervene.](image)

Cells contain HMGB1 in the nucleus, bound to nucleosomes. HMGB1 can be secreted by the activation of cells or passively by apoptosis or necrosis. Outside the cell, HMGB1 can bind to inflammatory mediators and subsequently to receptors such as TLR2, TLR4 and RAGE, leading to cell activation and proliferation. These actions can be inhibited by using anti-HMGB1 antibodies or by drugs that promote nuclear retention of HMGB1.
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(IFNRI). These double knock-out mice spontaneously develop chronic, destructive polyarthritis which shares many features with rheumatoid arthritis. Mice were injected with either the antagonist Box A or anti-HMGB1 antibodies for five weeks. A protective effect on joint destruction was shown and the clinical course of the arthritis was ameliorated (29,30). Taken together the studies described above demonstrate the proof of concept that HMGB1 blockade, either through already existing drugs, like gold salts or oxaliplatin or via experimental procedures specifically targeting HMGB1, can mediate beneficial effects on arthritis development.

Endotoxemia

Mice exposed to lethal levels of endotoxin (LPS) die within a couple of days. In this process HMGB1 was identified as a late mediator of lethality, in contrast to TNFα or IL-1 which are considered early mediators. Mice showed increased serum levels of HMGB1 from 8 to 32 hours after endotoxin exposure (3). Nontoxic doses of HMGB1 administered together with non-lethal doses of LPS acted in synergy to cause toxicity, indicating that HMGB1 played a critical role in lethality. Furthermore, treatment with anti-HMGB1 antibodies significantly improved survival, even when given after LPS injection (3). Metformin, a known anti-diabetic drug, has been shown to beneficially influence the survival of LPS-injected mice by systemic inhibition of HMGB1 release. In contrast, no improved survival could be observed when mice were injected with HMGB1 and metformin (31). Another compound that caused retention of HMGB1 from LPS-stimulated macrophages is DIC (3-(3-chlorophenyl)-5-(4-pyridyl)-4,5-dihydroisoxazole) (32). This recently discovered new anti-inflammatory compound was also shown to inhibit TNF-α and IL-6 release after LPS stimulation. However, more research is needed to fully investigate the potential therapeutic value of this compound.

Sepsis

A representative mouse model of sepsis is induced by infection with replicating bacteria, using cecal ligation and puncture (CLP). Similar to the endotoxin model, the administration of anti-HMGB1 antibodies or the Box A antagonist increased survival in this model (33). Furthermore, it was shown that anti-HMGB1 antibodies also have positive effects on the cognitive impairment of sepsis survivors (34). Glucan phosphate (GP) attenuates cardiac dysfunction and increases survival in CLP-induced septic mice by nuclear sequestering of HMGB1. Inhibition of NF-κB, an important transcription factor in the TLR4 signaling pathway, reduced HMGB1 translocation. Furthermore, TLR4 expression is upregulated in CLP mice, while treatment with GP attenuated CLP-induced increases in TRL4 expression (35).
Another model which can be used for systemic inflammation is a model of crush injury. Patients with crush injury often present with systemic inflammatory response syndrome and develop multiple organ failure. In this model both hindlimbs of rats are compressed for 6 h and then released. Afterwards the levels of HMGB1 in the sera of these rats were elevated. Treatment with anti-HMGB1 antibodies improved survival and lowered cytokine levels. Moreover, 24 hours after crush injury there was decreased RAGE expression in treated animals compared to untreated animals (36).

**Ischemia/reperfusion injury**
Ischemia-reperfusion injury is a direct, adverse consequence of transplantation, which can negatively impact both short- and long-term graft survival. The initial sterile injury leads to activation of the innate immune system and results in the release of cytokines which mediate the development of systemic inflammatory responses and local tissue damage. HMGB1 is released as an early mediator of ischemia-reperfusion injury and is shown to translocate from the nucleus to the cytoplasm, particularly in vascular and tubular cells (37-41). In two models of renal ischemic injury the beneficial effects of blocking HMGB1 were demonstrated both showing improved renal function (37,39) albeit by different methods. One study used ethyl pyruvate which causes nuclear retention of HMGB1 (37) while another employed neutralizing antibodies against HMGB1 (39). Also, in a model of intestinal ischemia-reperfusion injury serum HMGB1 levels were found to be increased and peaked 3 hours after ischemia-reperfusion injury. Moreover, a high degree of positive HMGB1 staining was observed in the epithelial cells of the damaged villi compared to the sham treated group. In this model, anti-HMGB1 antibodies increased survival and significantly reduced the damage to the villi (41).

**CONCLUSIONS**
HMGB1 has been shown to play an important role as an inflammatory mediator, which can act alone or in complex with several other molecules. Much knowledge has already been amassed by in vitro studies which investigate the pathways, receptors and posttranslational modifications of HMGB1. As shown in this review and table 1, this knowledge has been used in pre-clinical studies in several different animal models using blocking agents or neutralizing antibodies against HMGB1 to alleviate symptoms of disease. However, in order to use these agents as a therapeutic drug in humans more studies have to be performed. These studies should investigate how to specifically block the unwanted effects of HMGB1 while keeping in mind that both intracellular and extracellular HMGB1 have important roles in the early
Table 1: Experimental disease models using several different modes of therapies to target HMGB1

<table>
<thead>
<tr>
<th>Experimental model</th>
<th>Mode of therapeutic HMGB1 targeting</th>
<th>References</th>
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<tbody>
<tr>
<td>Arthritis</td>
<td>anti-HMGB1 monoclonal antibody</td>
<td>(29)</td>
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<tr>
<td></td>
<td>anti-HMGB1 polyclonal antibody</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td>Box A antagonist</td>
<td>(28) (30)</td>
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<tr>
<td></td>
<td>nuclear retention</td>
<td>(27) (24)</td>
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<tr>
<td>Endotoxemia</td>
<td>inhibition of HMBG-1 release</td>
<td>(31)</td>
</tr>
<tr>
<td>CLP</td>
<td>anti-HMGB1 monoclonal antibody</td>
<td>(34)</td>
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<tr>
<td></td>
<td>glucan phosphate (nuclear sequestering of HMGB1)</td>
<td>(35)</td>
</tr>
<tr>
<td>Crush injury/systemic inflammation</td>
<td>anti-HMGB1 polyclonal antibody</td>
<td>(36)</td>
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<tr>
<td>Ischemia reperfusion (kidney)</td>
<td>nuclear retention</td>
<td>(37)</td>
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<td></td>
<td>anti-HMGB1 polyclonal antibody</td>
<td>(39)</td>
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<tr>
<td>Ischemia reperfusion (intestine)</td>
<td>anti-HMGB1 polyclonal antibody</td>
<td>(41)</td>
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<tr>
<td>Hepatoxicity</td>
<td>anti-HMGB1 polyclonal antibody</td>
<td>(42)</td>
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<tr>
<td>Pain</td>
<td>systemic injection of glycyrrhizin (binds to HMGB1)</td>
<td>(43) (45)</td>
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<td></td>
<td>anti-HMGB1 polyclonal antibody</td>
<td>(44) (45)</td>
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<tr>
<td>Islet transplantation</td>
<td>anti-HMGB1 monoclonal antibody</td>
<td>(46)</td>
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<tr>
<td>Atherosclerosis</td>
<td>anti-HMGB1 monoclonal antibody</td>
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<td>Ischemia (brain)</td>
<td>siRNA for HMGB1</td>
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<td></td>
<td>anti-HMGB1 monoclonal antibody</td>
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<tr>
<td>Brain injury/damage to the blood</td>
<td>Box A antagonist</td>
<td>(50)</td>
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<tr>
<td>brain barrier</td>
<td>anti-HMGB1 monoclonal antibody</td>
<td>(51)</td>
</tr>
<tr>
<td>Intestinal tissue damage</td>
<td>anti-HMGB1 polyclonal antibody, nuclear retention</td>
<td>(52)</td>
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Detection of invasion by pathogens and injury that culminates in activating innate immunity. It has been recently shown that it is possible to block specific actions of HMGB1 with different molecules. The Box A antagonist for instance has been shown to only block the chemo attractant form of HMGB1 without interfering with the cytokine inducing activity (10). Thus it is conceivable that the therapeutic effects of Box A described in the studies in this review can be entirely attributed to reduced recruitment of inflammatory cells in the inflamed tissue. For the different monoclonal and polyclonal antibodies used it should be investigated whether the beneficial effects of HMGB1 blocking can be attributed to chemoattractant or cytokine inhibitory properties. Lastly, side-effects and long-term analysis also have to be investigated; most animal studies tend to run for only a couple of weeks to
investigate the direct effects of HMGB1. For diseases like sepsis and ischemia/reperfusion this poses no problem, as those are acute diseases. In contrast, RA and many other autoimmune diseases are chronic and thus future research has to focus on the long term effects of HMGB1 blocking. The study using oxaliplatin to treat the collagen type II induced arthritis in mice was the only one to report a relapse, which occurred one week after injection with oxaliplatin. The relapse coincided with the presence of extracellular HMGB1, and was associated with an increase incidence of arthritis in the treated animals. The mechanism behind this relapse was not investigated, although it was shown that there was no difference in the presence of apoptotic and necrotic cells in the treated and untreated animals (27). Thus more studies, delineating the kinetics and pharmacological properties of HMGB1 should be performed. Hopefully, it will be possible in the future to translate the progress made in animal studies into therapeutics for clinical trials in humans.

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Conflict of interest: The authors state no conflict of interest.

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