High Mobility Group Box 1 in the Pathogenesis of Inflammatory and Autoimmune Diseases

Reinhard E. Voll MD[1,2], Vilma Urbonaviciute[1], Martin Herrmann PhD[2] and Joachim R. Kalden MD[2]

[1]IZKF research group 2, Nikolaus-Fiebiger-Center of Molecular Medicine, University Hospital Erlangen, Erlangen, Germany
[2]Department of Internal Medicine 3, University Hospital Erlangen, Erlangen, Germany

Key words: HMGB1, nucleosomes, autoantibodies, autoimmunity, inflammation, systemic lupus erythematosus

Abstract

High mobility group box 1 is a nuclear protein participating in chromatin architecture and transcriptional regulation. When released from cells, HMGB1 can also act as a pro-inflammatory mediator or alarmin. Upon stimulation with lipopolysaccharides or tumor necrosis factor-alpha, HMGB1 is secreted from certain cells such as monocytes/macrophages and fosters inflammatory responses. In addition, HMGB1 is passively released from necrotic cells and mediates inflammation and immune activation. In contrast, during apoptotic cell death, nuclear HMGB1 becomes tightly attached to hypo-acetylated chromatin and is not released into the extracellular milieu, thereby preventing an inflammatory response. There is accumulating evidence that extracellular HMGB1 contributes to the pathogenesis of many inflammatory diseases, including autoimmune diseases. Increased concentrations of HMGB1 have been detected in the synovial fluid of patients with rheumatoid arthritis. In animal models of RA, HMGB1 appears to be crucially involved in the pathogenesis of arthritis since neutralization of HMGB1 significantly ameliorates the disease. Also, in the serum and plasma of patients with systemic lupus erythematosus we detected substantial amounts of HMGB1, which may contribute to the disease process. However, investigations of blood concentrations of HMGB1 and its relevance in human diseases are hindered by the lack of reliable routine test systems.

IMAJ 2008;10:26–28

High mobility group box 1 protein is a ubiquitously expressed and evolutionarily conserved chromosomal protein. HMGB1 contains two positively charged DNA binding domains termed HMG box A and B, and a negatively charged C-terminal domain containing 30 repetitive glutamic and aspartic acid residues [1,2]. HMGB1 binds to double-stranded, single-stranded as well as distorted DNA without sequence specificity [2-6]. In addition, HMGB1 interacts with high affinity with nucleosomes, stabilizes their structure, and mediates bending of the DNA, thus facilitating the binding of certain transcription factors including steroid hormone receptors [7].

Studies in endotoxemia and sepsis showed that extracellular HMGB1 can act as a pro-inflammatory cytokine, which is actively secreted from lipopolysaccharide- or tumor necrosis factor-activated macrophages/monocytes, pituicytes and other cells. Importantly, HMGB1 appears to mediate late lethality from endotoxic shock [8-12]. In addition, HMGB1 is passively released from necrotic cells, inducing an inflammatory response. In contrast, apoptotic cells retain HMGB1, which is tightly attached to hypo-acetylated chromatin. Therefore, HMGB1 is not released from apoptotic cells and does not induce inflammation [9,13].

Extracellular HMGB1 binds to cell surface receptors such as the receptor for advanced glycation end products, Toll-like receptors 2 and 4 and, possibly, to yet unknown receptors [7,9,14]. Receptor binding leads to activation of the transcription factor nuclear factor-kappa B, inducing the transcription of multiple pro-inflammatory genes. Upon (co-)activation with HMGB1, macrophages produce pro-inflammatory cytokines such as TNFα, interleukin-1β, IL-6, IL-8, macrophage inflammatory protein-1α and MIP-2β [15]. In addition, HMGB1 induces activation/maturation of dendritic cells with expression of major histocompatibility class II, CD83, CD80 and CD86 [9, 16].

There is increasing evidence that HMGB1 contributes to the pathogenesis of chronic inflammatory and autoimmune diseases due to its pro-inflammatory and immunostimulatory properties. Elevated levels of extracellular HMGB1 have been reported in experimental arthritis models. Similarly, in humans with rheumatoid arthritis increased concentrations of HMGB1 were detected within the synovial fluid from inflamed joints [9,17,18]. In contrast to reports of HMGB1 in synovial fluid, we did not detect elevated concentrations of HMGB1 within serum or plasma of patients with RA [19]. Importantly, collagen-induced arthritis in rodents was significantly ameliorated upon systemic application of either an antagonistic A box domain or neutralizing HMGB1-specific antibodies, indicating an important role in the pathogenesis of arthritis [17,19].

Within the lesional skin of cutaneous lupus erythematosus, increased amounts of cytoplasmic and extracellular HMGB1 have been detected, together with high expression of TNFα and IL-1β [20]. A follow-up investigation demonstrated cytoplasmic and extracellular HMGB1 at the peak of clinical activity in experimentally ultraviolet-induced lesions of cutaneous lupus [21].

Using Western blot analysis and enzyme-linked immunosor-

HMGB1 = high mobility group box 1 protein
RA = rheumatoid arthritis

TNFα = tumor necrosis factor-alpha
IL = interleukin
MIP = macrophage inflammatory protein

bent assay we found increased concentrations of HMGB1 in the serum and plasma of a substantial number of patients with SLE [19]. Importantly, HMGB1 appears to be attached to circulating nucleosomes, most likely released from secondary necrotic cells [Urbonaviciute et al., submitted].

We propose the following model in which HMGB1 plays a crucial role in the immunopathogenesis of SLE. Normally, apoptotic cells are cleared swiftly in the early phases of apoptosis by phagocytes. As a result, apoptotic cells display a potent anti-inflammatory and immunosuppressive effect on monocytes/macrophages [22,23] that may prevent autoimmunity. However, in approximately 40% of SLE patients the phagocytosis of dead cells is impaired both *in vitro* and *in vivo* [24,25]. Hence, dying cells may enter late stages of apoptosis, i.e., secondary necrosis, and release HMGB1-containing nucleosomes since HMGB1 is tightly attached to the chromatin of apoptotic cells [13]. Nucleosomes as ubiquitously expressed abundant cellular components should establish profound central and peripheral tolerance, explaining their low immunogenicity under normal conditions. However, according to our recent data, HMGB1 complexed to "apoptotic" nuclesomes can activate dendritic cells and macrophages, thereby contributing to breaking the immunological tolerance to nucleosomes and double-stranded DNA, which represent key antigens in SLE [Urbonaviciute et al., submitted].

HMGB1 itself can be the target of an autoimmune response: anti-HMGB1 antibodies were found in patients with several autoimmune diseases including systemic sclerosis, ulcerative colitis, juvenile idiopathic arthritis, and SLE. However, the clinical relevance of these findings remains to be determined [13,26-28]. We also identified immunoglobulin G and M antibodies to HMGB1 in patients with SLE. Unexpectedly, most healthy blood donors displayed detectable amounts of anti-HMGB1 antibodies in their serum, although in lower concentrations than those of SLE patients. The presence of low titers of anti-HMGB1 antibodies in the majority of healthy subjects might be due to cross-reactivity or the sticky nature of HMGB1 [19]. HMGB1-binding antibodies might have physiological relevance by modulating the pro-inflammatory activity of HMGB1, thereby limiting overwhelming inflammatory responses caused by massive HMGB1 release in conditions such as sepsis and extensive necrosis. Importantly, anti-HMGB1 autoantibodies impede the reliable quantification of HMGB1 by ELISA [19]. Hence, the development of routine diagnostic methods for the reliable quantification of HMGB1 in serum and plasma is required to further elucidate the role of this multifunctional protein as a diagnostic and/or prognostic marker and as a potential therapeutic target in immune and inflammatory diseases.

Acknowledgment. Supported by the Interdisciplinary Center for Clinical Research (IZKF) at the University Hospital of the University of Erlangen-Nuremberg, project N2.

SLE = systemic lupus erythematosus
ELISA = enzyme-linked immunosorbent assay

References

1. Tsuda K, Kikuchi M, Mori K, Waga S, Yoshida M. Primary structure of non-histone protein HMG1 revealed by the nucleotide sequence. *Biochemistry* 1988;27:6159–63.
2. Thomas JO, Travers AA. HMG1 and 2, and related 'architectural' DNA-binding proteins. *Trends Biochem Sci* 2001;6:167–74.
3. Bustin M. Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. *Mol Cell Biol* 1999;19:5237–46.
4. Bustin M, Reeves R. High-mobility-group chromosomal proteins: architectural components that facilitate chromatin function. *Prog Nucleic Acid Res Mol Biol* 1996;54:35–100.
5. Bianchi ME, Beltrame M, Paonessa G. Specific recognition of cruciform DNA by nuclear protein HMG1. *Science* 1989;243:1056–9.
6. Bianchi ME, Falciola L, Ferrari S, Lilley DM. The DNA binding site of HMG1 protein is composed of two similar segments (HMG boxes), both of which have counterparts in other eukaryotic regulatory proteins. *Embo J* 1992;11:1055–63.
7. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* 2005;5:331–42.
8. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* 2007;81:1–5.
9. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999;285:248–51.
10. Chen G, Li J, Ochani M, et al. Bacterial endotoxin stimulates macrophages to release HMGB1 partly through CD14- and TNF-dependent mechanisms. *J Leukoc Biol* 2004;76:994–1001.
11. Rendon-Mitchell B, Ochani M, Li J, et al. IFN-gamma induces high mobility group box 1 protein release partly through a TNF-dependent mechanism. *J Immunol* 2003;170:3890–7.
12. Wang H, Vishnubhakat JM, Bloom O, et al. Proinflammatory cytokines (tumor necrosis factor and interleukin 1) stimulate release of high mobility group protein-1 by pituicytes. *Surgery* 1999;126:389–92.
13. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002;418:191–5.
14. Park JS, Svetkauskaite D, He Q, et al. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. *J Biol Chem* 2004;279:7370–7.
15. Andersson U, Wang H, Palmblad K, et al. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. *J Exp Med* 2000;192:565–70.
16. Rovere-Querini P, Capobianco A, Scaffidi P, et al. HMGB1 is an endogenous immune adjuvant released by necrotic cells. EMBO *Rep* 2004;5:825–30.
17. Kokkola R, Sundberg E, Ulfgren AK, et al. High mobility group box chromosomal protein 1: a novel proinflammatory mediator in synovitis. *Arthritis Rheum* 2002;46:2598–603.
18. Taniguchi N, Kawahara K, Yone K, et al. High mobility group box chromosomal protein 1 plays a role in the pathogenesis of rheumatoid arthritis as a novel cytokine. *Arthritis Rheum* 2003;48:971–81.
19. Urbonaviciute V, Furnrohr BG, Weber C, et al. Factors masking HMGB1 in human serum and plasma. *J Leukoc Biol* 2007;81:67–74.
20. Popovic K, Ek M, Espinosa A, et al. Increased expression of the novel proinflammatory cytokine high mobility group box chromosomal protein 1 in skin lesions of patients with lupus erythematosus. *Arthritis Rheum* 2005;52:3639–45.
21. Barkauskaite V, Ek M, Popovic K, Harris HE, Wahren-Herlenius M, Nyberg F. Translocation of the novel cytokine HMGB1 to the cytoplasm and extracellular space coincides with the peak of clinical activity in experimentally UV-induced lesions of cutaneous lupus erythematosus. *Lupus* 2007;16:794–802.

22. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. N*ature* 1997; 390:350–1.
23. Fadok VA, McDonald PP, Bratton DL, Henson PM. Regulation of macrophage cytokine production by phagocytosis of apoptotic and post-apoptotic cells. B*iochem Soc Trans* 1998;26:653–6.
24. Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR. Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. A*rthritis Rheum* 1998;41:1241–50.
25. Baumann I, Kolowos W, Voll RE, Manger B, et al. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. A*rthritis Rheum* 2002;46:191–201.
26. Ayer LM, Senecal JL, Martin L, Dixon GH, Fritzler MJ. Antibodies to high mobility group proteins in systemic sclerosis. J R*heumatol* 1994;21:2071–5.
27. Rosenberg AM, Cordeiro DM. Relationship between sex and antibodies to high mobility group proteins 1 and 2 in juvenile idiopathic arthritis. J R*heumatol* 2000;27:2489–93.
28. Sobajima J, Ozaki S, Uesugi H, et al. Prevalence and characterization of perinuclear anti-neutrophil cytoplasmic antibodies (P-ANCA) directed against HMG1 and HMG2 in ulcerative colitis (UC). C*lin Exp Immunol* 1998;111:402–7.

Correspondence: Dr. R.E. Voll, IZKF Research Group 2, Nikolaus-Fiebiger-Center, Glückstrasse 6, 91054 Erlangen, Germany.
Phone: (49-9131) 853-9301
Fax: (49-9131) 853-9311
email: rvoll@molmed.uni-erlangen.de