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REVIEW

High-mobility group box 1 (HMGB1) as a master regulator of innate immunity

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Abstract Damage-associated molecular patterns (DAMPs) comprise intracellular molecules characterized by the ability to reach the extracellular environment, where they prompt inflammation and tissue repair. The high-mobility box group 1 (HMGB1) protein is a prototypic DAMP and is highly conserved in evolution. HMGB1 is released upon cell and tissue necrosis and is actively produced by immune cells. Evidence suggests that HMGB1 acts as a key molecule of innate immunity, downstream of persistent tissue injury, orchestrating inflammation, stem cell recruitment/activation, and eventual tissue remodeling.

Keywords High-mobility group box 1 (HMGB1) · Innate immunity · Damage-associated molecular patterns (DAMPs)

Introduction

Innate immunity encompasses an immediate and stereotypical response to diverse events that share the potential to jeopardize cell and tissue integrity. Immunologists have, until recent years, focused on the innate response elicited by microorganisms. Protection against invading microbes relies on the recognition of the molecular structures shared by pathogens, referred to as pathogen-associated molecular patterns (PAMPs; Janeway 1992). Dedicated pattern-recognition receptors (PRRs) recognize PAMPs and thus allow them to identify pathogens. Most innate immune cells express PRRs. PRR activation in turn recruits several signaling pathways. As a consequence, PAMP recognition:

(1) promotes the production of soluble inflammatory molecules, including cytokines and chemokines, which recruit and locally activate inflammatory leukocytes (Nathan 2002), with the inflammatory cells acquiring the ability to terminate the pathogen and any cells that had been infected;
(2) elicits an acute phase response, with the generation and release of conserved soluble pattern recognition receptors (Manfredi et al. 2008a), which locally tune and regulate the potentially noxious effects of leukocyte activation;
(3) favors the migration of antigen-presenting cells, in particular dendritic cells (DCs), to secondary lymphoid organs, where they productively activate naive T cells. The clonal expansion of antigen-specific T cells is the basis of an adaptive immunological response, characterized by specificity for the pathogen and by memory; expanded clones retain the ability to react faster and more effectively in the case of a further encounter with the microbe on future occasions (Pulendran et al. 2001). The effect of PAMP recognition is not only quantitative; depending on the specific array of PRRs, antigen presentation in lymphoid organs results in the preferential expansion of lymphocytes committed toward a Th1, Th2, Th17, or regulatory T-cell fate, therefore better suited to enforce protective immunity effective against diverse microbes, to promote inflammation and autoimmunity, or to establish tolerance (Manfredi et al. 2009).

Sterile inflammation, which occurs as a consequence of tissue necrosis, even in the absence of pathogens, allows effective clearance of necrotic cells and debris, thus preventing the dangers (Matzinger 2002) associated to residual uncleared material (Munoz et al. 2010a) and enforcing

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effective regeneration programs, with eventual tissue healing. Inflammation, in particular, sustains the secretion of growth and survival factors by bystander tissue cells that have not been directly damaged; this in turn recruits and activates local precursor and stem cells (Shaw and Martin 2009). Novel extracellular matrix assembly, via deposition by fibroblasts and degradation by activated macrophages, further contributes to eventual healing. Complex syndromes, including generalized sterile inflammation and systemic inflammatory response syndrome on one hand, and possibly specific features of autoimmune systemic rheumatic diseases, represent part of the price for the protection that inflammation provides (Banchereau and Pascual 2006; Pisetsky et al. 2008; Hreggvidsdottir et al. 2009; Pisetsky and Ronnblom 2009). Specifically, damage-associated molecular patterns (DAMPs) or alarmins are intracellular molecules that are released during cell and tissue necrosis and are endowed with the ability to elicit inflammation and, possibly as a direct consequence, both tissue regeneration and activation of acquired T-cell-dependent protective immune (and autoimmune) responses (Oppenheim and Yang 2005; Lotze et al. 2007; Bianchi and Manfredi 2009; D. Yang et al. 2010).

In real-life, sterile injuries readily become infected. Conversely, infection is associated with cell death and therefore with the release of DAMPs, with the specific features and extent of the inflammatory response. Here, we discuss, in particular, the ability of the best-characterized endogenous DAMP, the high-mobility group box 1 protein (HMGB1), to contribute to the innate responses in injured and/or infected peripheral tissues. HMGB1 is mostly located in the nucleus of living cells. Its structure (Fig. 1) justifies its efficacy at bending DNA, thus promoting the assembly of proteins on specific targets. Moreover, it is possibly involved in the ability of HMGB1 to form biologically active complexes with diverse substrates, which are heterogeneous in terms of physico-chemical and biological properties (see below).

The HMG family

HMGB1 is an abundantly occurring parent form of HMG proteins. Its name indicates its ability to migrate quickly in Triton-urea and polyacrylamide gels, a feature that reflects the high content of charged amino acid residues and that interestingly also reflects its "temperament" in the nucleus of living cells, where it is highly motile. *Hmgb1* is located on the 13q12 human chromosome. The gene consists of six exons that encode for a 215-amino-acid polypeptide. It has a large sequence consensus in all animal species (Sessa and Bianchi 2007). Mammals have several genes, including *Hmgb1*, *Hmgb2*, and *Hmgb3*, which express similar (>80% identity) proteins. They code for proteins with a molecular mass of around 25 kDa, two DNA-binding domains (referred to as box A and box B), and a long acidic carboxy-terminal region. HMGB proteins are not redundant, as demonstrated by triple HMGB silencing (Yanai et al. 2009). HMGB1 proteins from all mammals are virtually identical (>99%), implying that each single residue is under selective pressure; HMGB2 and HMGB3 are also strongly conserved (Stros 2010). A shorter HMGB4 protein, devoid of the acidic tail and possibly endowed with specific nuclear roles, has also been recently identified (Catena et al. 2009).

HMGB1 has been extensively studied during the last two decades. So far, other members of the family have received less attention. Embryos express high levels of both HMGB1 and HMGB2 proteins. In contrast, HMGB1 is expressed in almost all nucleated cells of adult animals (and not only adults; see Rouhiainen et al. 2000) and HMGB2 in testis and lymphoid organs (Muller et al. 2004). Moreover, HMGB2 is expressed in the superficial zone of articular cartilage, where it plays a protective role during aging (Taniguchi et al. 2009). HMGB2 has been further characterized during the last two years as a chromatin protein (Stros 2010; Taniguchi et al. 2009; Ugrinova et al. 2009; Tian et al. 2007; Lee et al. 2010). Moreover, HMGB2 plays a role in cell death and inflammation (Pusterla et al. 2009; Krynetskaia et al. 2009; Wixted et al. 2010) thanks to its mitogenic and chemoattractant activities (Pusterla et al. 2009) and to its putative involvement in the response to oxidative stress (Lee et al. 2010). HMGB proteins 1, 2, and 3 share the ability to bind to nucleic acids and are required for type-I interferon and inflammatory cytokine induction by DNA or RNA (Yanai et al. 2009; see also below).

HMGB1: a molecule in motion

The positively charged DNA-binding domains (A and B boxes) of HMGB1 contain nuclear-localization signals. Interestingly, they seem to have distinct extra-cellular functions: the A box is an antagonist of B box pro-inflammatory activity (Li et al. 2003). The tail specifically interacts with the two boxes and influences their ability to bind to DNA (Knapp et al. 2004).

HMGB1 is ubiquitously expressed; its level and subcellular localization depend on the cell type and state of activation, with more differentiated cells often being characterized by a lower content of the protein (Muller et al. 2004). HMGB1 expression is mostly nuclear; however, under certain conditions, the molecule reaches the cytoplasm and thereafter the extracellular environment. Schematically, HMGB1 leaves the nucleus either because:

(1) cells die via an unscheduled accidental pathway that is associated with the loss of membrane compartmentalization and with the release of intracellular components;

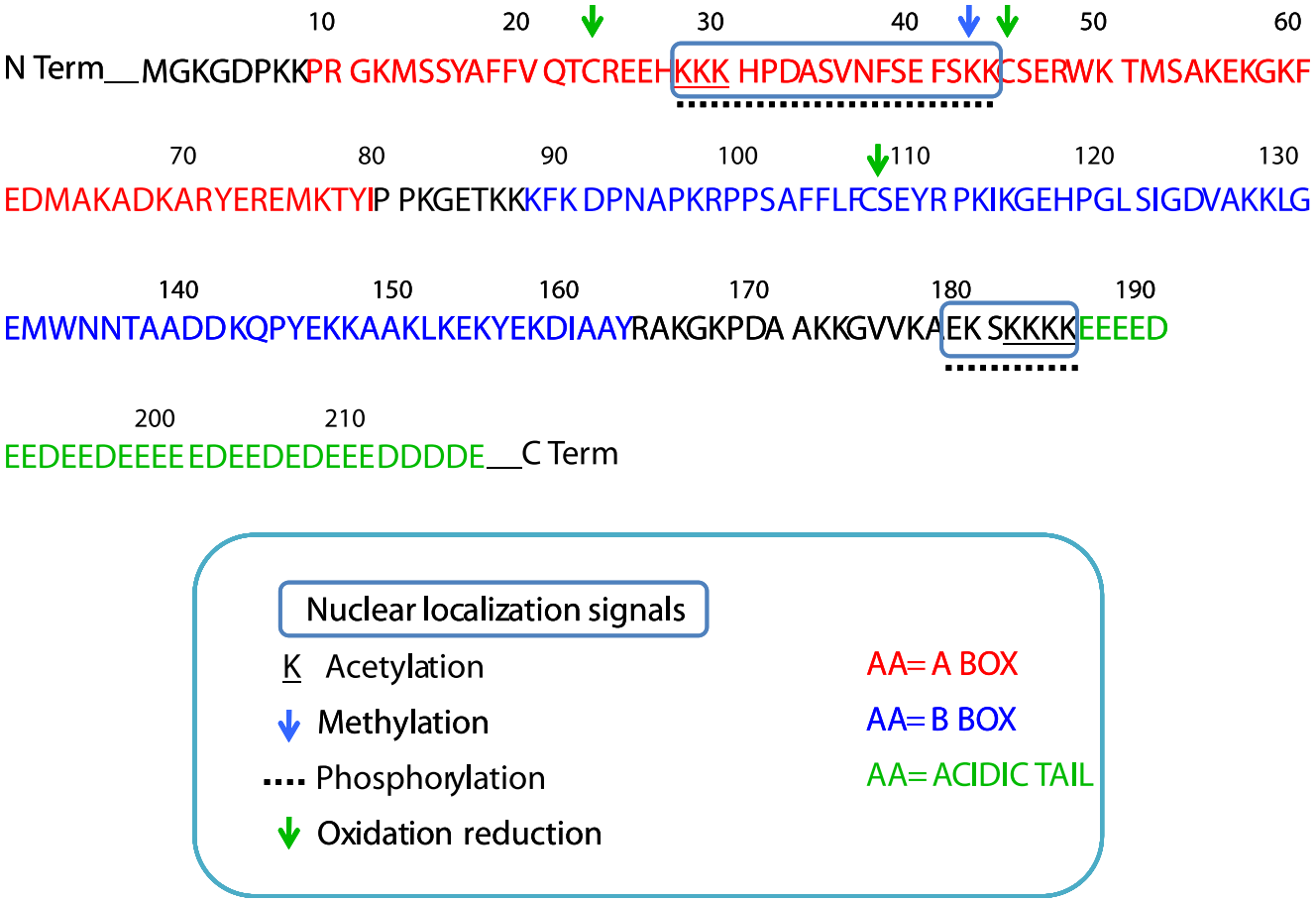

Fig. 1 High-mobility group box 1 (HMGB1) domains and putative targets for post-translational modifications

(2) cells undergo activation and actively secrete HMGB1. During innate immune responses, leukocytes secrete intracellular components, thus artificially recreating the environment associated with cell necrosis, a primeval condition associated with immune activation (Bianchi and Manfredi 2004, 2007).

Monocytes, macrophages and immature DCs secrete HMGB1 in response to lipopolysaccharide (LPS), tumor necrosis factor α (TNFα), or interleukin-1β (IL-1β) stimulation (Wang et al. 1999; Dumitriu et al. 2005a, b, c). Post-translational modifications, including acetylation, phosphorylation, methylation, and oxidation influence the function of extracellular HMGB1 (Gardella et al. 2002; Bonaldi et al. 2003; Hoppe et al. 2006; Youn and Shin 2006; Ito et al. 2007; see also below).

Acetylated lysines at positions 2 and 11 are a feature of HMGB1 released by dying cells. In contrast, lysines throughout the entire length of actively secreted HMGB1 are acetylated (Bonaldi et al. 2003), including those within the 27–43 and 178–184 domains that behave as nuclear localization signals. Acetylation might influence the intracellular localization of the HMGB1 in activated cells, facilitating access to "secretory" lysosomes, a group of intracellular vesicles whose content is released into the extracellular environment in the presence of appropriate secretagogs (Bianchi and Manfredi 2007; Wang et al. 1999; Dumitriu et al. 2005a, b, c; Gardella et al. 2002). This pathway is apparently dominant in myeloid cells. Other cells, including neurons, also actively secrete HMGB1 in the absence of *bona-fide* secretory lysosomes (Rauvala and Rouhiainen 2010). Other modifications, including acetylation and phosphorylation possibly facilitate nuclear/cytoplasmic shuttling (Youn and Shin 2006). Phosphorylation is calcium-dependent and is mediated by the classical protein kinase C (Oh et al. 2009). Mono-methylation of lysine at position 42 also occurs post-translationally. Methylated HMGB1 is less effective at DNA binding and, apparently as a consequence, passively diffuses in the cytoplasm of neutrophils (Ito et al. 2007).

A redox-regulated biological function?

HMGB1 function in the environment depends on its functional integrity. In turn, environmental cues directly target and modify HMGB1. Oxidative stress is an early player during acute inflammatory response and results in

the formation of reversible covalent disulfide bonds between thiols (Rubartelli and Sitia 2009; Carta et al. 2009). HMGB1 contains three cysteine residues at positions 23, 45, and 106. Upon mild oxidation, cysteines at positions 23 and 45 establish an intra-molecular disulfide bridge, which is reverted under reducing conditions (Hoppe et al. 2006). In contrast, the cysteine at position 106 contributes to the nuclear localization of the molecule (Hoppe et al. 2006). Moreover, the residue is required for binding to the Toll-like receptor 4 (TLR4) PRR on the macrophage plasma membrane and for HMGB1-elicited cytokine secretion (H. Yang et al. 2010). So far, the precise effect of the redox state on this interaction has not, to the best of our knowledge, been investigated.

The latter issue is relevant; a regulated change of the intra- and extra-cellular redox state characterizes two events in which HMGB1 plays a key role, i.e., cell death and inflammation. Necrotic cells are a primary source of HMGB1 (Scaffidi et al. 2002; Raucci et al. 2007). HMGB1 released by necrotic cells appears to be oxidized: the molecular pathway by which oxidation takes place has not so far been elucidated (Urbonaviciute et al. 2009). Eventually, HMGB1 also undergoes oxidation in cells that die via apoptosis. Apoptosis is associated with the generation of reactive oxygen species (ROS) by mitochondria, which in turn oxidize the cytosine at position 106 (Kazama et al. 2008). As a consequence, HMGB1 extracellular functions are dramatically altered. This aspect has been studied verifying, in particular, the ability of the molecule to activate or tolerate the acquired immune response (Kazama et al. 2008), a feature that primarily depends on the action of HMGB1 on DCs (Manfredi et al. 2009; Dumitriu et al. 2007; Yang et al. 2007). This is of particular importance, since HMGB1 associates with nucleosomes that are generated during apoptotic cell death and that represent a key autoantigen in systemic autoimmunity (Urbonaviciute et al. 2008; Munoz et al. 2010b). The central role of HMGB1 in autoimmune diseases has been the topic of excellent recent reviews (Pisetsky et al. 2008; Abdulahad et al. 2010; Andersson and Harris 2010; Pisetsky 2010) and will not be discussed further here.

ROS generation is a common occurrence in living cells. In particular it occurs after activation of PRRs expressed by inflammatory cells. As a consequence, antioxidant responses are activated, which contribute to limit excessive oxidation of the inflamed environment (Rubartelli and Sitia 2009; Carta et al. 2009). The net effect of oxidant and antioxidant events might be important, given the exquisite sensitivity of HMGB1 to oxidation. An oxidized environment by inactivation of HMGB1 has been proposed to restrict the action of the molecule both temporally and spatially, thus focusing it when and where it is needed. Conversely, a reduced environment might contribute to maintaining and prolonging HMGB1 bioactivity (Carta et al. 2009). An interesting feedback loop has been recently identified: HMGB1 promotes the survival and the activation of eosinophils, thus possibly providing a molecular explanation for their preferential recruitment within necrotic tissues. In turn, eosinophils respond to HMGB1 with an oxidative burst, and the generated gaseous species inactivate HMGB1 (Lotfi et al. 2009) possibly limiting the immunogenicity of antigens associated with necrotic tissues, including specifically tumor-associated antigens. Further support for a role of the redox potential in finely tuning the extracellular actions of HMGB1 is provided by data on the role of apurinic/apyrimidinic endonuclease 1/Redox factor-1 (APE1), a multifunctional protein that regulates the reduction-oxidation balance on HMGB1 release and on events downstream of HMGB1 recognition, including the activation of p38 and c-Jun N-terminal kinase, ROS generation, cytokine secretion, and cyclooxygenase-2 expression by monocytes and macrophage-like cells (Yuk et al. 2009).

Bound (or unbound) HMGB1

HMGB1 *per se* is well established as having mitogenic and chemoattractive properties (Rouhiainen et al. 2007). Moreover, HMGB1 triggers the release of cytokines from inflammatory leukocytes, although the pro-inflammatory effect of the recombinant molecule has been discussed (Rouhiainen et al. 2007). The reasons for these discrepancies have not so far been identified. Post-translational modification of the molecule, depending on the characteristics of the cells or on the environmental conditions (see above), could well yield molecules endowed with only partially overlapping extracellular functions.

The issue may be even more complex in vivo. HMGB1 is a molecule that "loves company" (Bianchi 2009) and has promiscuous habits. It forms relatively stable multimolecular complexes with various substrates molecules. Some ligands *per se* interact, on cells, with receptors of the innate immunity system, including nucleic acids, PAMPs, and selected cytokines and chemokines. HMGB1-containing complexes are likely to be more the rule than an exception in inflamed tissues, being by definition characterized by the presence of cytokines, of microbial products, and of by-products of dying and activated cells. HMGB1 association stabilizes and complements the biological function of its substrate via the simultaneous or sequential activation of various PRRs (see below).

HMGB1 and LPS physically interact (Hreggvidsdottir et al. 2009; Youn et al. 2008). The complexes elicit the release of inflammatory cytokines more effectively than either molecule alone (Youn et al. 2008). HMGB1 therefore has

the potential to act at inflammatory sites as a potent endogenous amplificatory signal, endowed with the ability to magnify the effects even of traces of bacterial components. Interestingly, separate A box and B box HMGB1 domains bind to LPS and enhance IL-6 production (Hreggvidsdottir et al. 2009). Inflammatory endogenous molecules also associate with HMGB1: this is the case for IL-1β (Sha et al. 2008). As described for LPS, HMGB1/IL-1β complexes are more effective than IL-1β alone and elicit a higher production of IL-6 (Hreggvidsdottir et al. 2009), of major intrinsic protein-2 and TNFα (Sha et al. 2008). The activity of HMGB1/IL-1β complexes is inhibited by adding, separately, neutralizing antibodies for the cytokine and its receptor, indicating that the complex acts through the IL-1β receptor. The actual mechanism(s) by which HMGB1 enhances and prolongs the activity of IL-1β, and possibly also of TNFα and interferon γ (Sha et al. 2008), has(have) not so far been identified.

The response to chemotactic signals is a critical issue in immunity; it requires that motile cells are recruited at inflammatory sites or reach lymphoid organs. HMGB1 is a crucial regulator of the fate and function of DCs (Dumitriu et al. 2005a, c; Andersson and Harris 2010; Rovere-Querini et al. 2004; Messmer et al. 2004; Semino et al. 2005; Ulloa and Messmer 2006; Apetoh et al. 2007a, b), professional antigen-presenting cells that connect innate and acquired immune responses. DCs, like most myeloid cells, translocate HMGB1 from the nucleus in the cytosol upon activation and eventually release the molecule into the extracellular environment (see above). Secreted HMGB1 is biologically active and required for DC maturation, a complex event by which activated DCs switch their responsiveness to chemokines from CCL5 to CCL21, thus acquiring the ability to migrate to the T cell zone of secondary lymph nodes (Randolph et al. 2005). Indeed, DCs acquire antigens in peripheral tissues and present them to T lymphocytes several hours later in the lymph nodes, i.e., at a distant site after a time-consuming journey. In the presence of inhibitors of HMGB1 or of one of its best-characterized receptors, the receptor for advanced glycated end-products (RAGE; see below), DCs activated with PAMPs or cytokines fail to mature (Dumitriu et al. 2005a, c). As a consequence, they fail to sustain T cell proliferation and survival and Th1 polarization (Dumitriu et al. 2005a, c), to migrate in response to the lymph-node chemokines CCL19 and CXCL12 (Dumitriu et al. 2007), and effectively to reach lymphoid organs in vivo (Manfredi et al. 2008b).

Conversely, maturing DCs that migrate in response to CXCL12 release HMGB1, which is required for CXCL12-dependent migration in vitro; the formation of complexes in the fluid phase between the two molecules maintains the conformation and function of CXCL12 in a reducing environment, such as that of the lymph-node. This is therefore possibly important for the attraction of antigen-presenting cells at the relevant sites at which T-cell-dependent immune responses begin (Campana et al. 2009). The regulation of the leukocyte recruitment in vivo clearly involves several steps, including the interaction between RAGE and leukocyte β2 integrins (Orlova et al. 2007). HMGB1 also up-regulates the expression and the sensitivity to TLR4 of maturing DCs; TLR4-dependent signaling on DC is required for HMGB1-mediated liver injury upon ischemia reperfusion (Klune et al. 2008; Tsung et al. 2005, 2007).

The intracellular function of HMGB1 strictly depends on its ability to bind to DNA. Therefore, unsurprisingly, nucleic acids represent a ligand for HMGB1, even outside the cell. Binding to HMGB1, as described for other substrates (see above), influences nucleic acid recognition by innate immune cells and, thus, their inflammatory properties. The chromatin of cells undergoing apoptosis undergoes extensive modifications that lead to a tight and long-lasting interaction with HMGB1, in stark contrast with the loose interaction of the molecule with the DNA of living or primary necrotic cells (Scaffidi et al. 2002). Apoptotic cells represent a source of HMGB1 and nucleosomes (Bell et al. 2006; Jiang et al. 2007; Pisetsky and Fairhurst 2007), which are released *per se* or as multimolecular complexes. Nucleosome/HMGB1 complexes can be traced in the blood of autoimmune patients and represent the unusual combination between an autoantigen (the nucleosome) and a natural adjuvant such as HMGB1, which is capable of conferring immunogenicity to antigenic soluble and particulate substrates (Rovere-Querini et al. 2004). Indeed, HMGB1/nucleosome complexes effectively activate innate immune cells, including DCs and macrophages in vitro, while triggering the production of anti-histone and anti-double-stranded DNA in experimental animals (Urbonaviciute et al. 2008). The complexes might therefore be involved in the original breakdown of tolerance associated with deregulated or uncleared apoptosis, which then fosters the development, in appropriate genetic backgrounds, of self-sustaining autoimmune diseases (Bondanza 2004; Mahoney and Rosen 2005; Bondanza et al. 2007; Rovere-Querini et al. 2007; Munoz et al. 2009, 2010a).

Nucleic acids have long been known to trigger the release of cytokines, including type 1 interferons and chemokines. This event is mediated by the activation of dedicated PRRs expressed either on the cell membrane or within the cell (Latz et al. 2004; Marshak-Rothstein and Rifkin 2007). The requirement of the HMGB proteins (see above) for the innate recognition of nucleic acids has been elegantly demonstrated by using genetic tools (Yanai et al. 2009), although the hierarchy of the associated molecular events needs to be characterized at molecular levels.

HMGB1 effectively binds to synthetic sequences containing unmethylated cytosine-guanine (CpG) dinucleotides. These oligonucleotides mimic hypomethylated microbial DNA, which exerts inflammatory and immunostimulatory actions mostly via the activation of endosomal TLR9. CpG containing sequences trigger the secretion of HMGB1 from macrophages and DCs; in turn, HMGB1 favors the access of the molecule to the receptor (Ivanov et al. 2007), thus magnifying the cytokine release downstream of TLR9 activation (Hreggvidsdottir et al. 2009; Yanai et al. 2009). The involvement of RAGE in TLR9-MyD88-mediated cytokine production has also been clearly defined (Tian et al. 2007).

HMGB1: uno, nessuno, centomila (one, none, one hundred thousand)

The plurality of effects of HMGB1 on innate immune cells, mediated via direct or indirect actions on multiple PRRs (Hreggvidsdottir et al. 2009; Rauvala and Rouhiainen 2010; H. Yang et al. 2010; Andersson et al. 2000; Sims et al. 2010; Fig. 2) that converge on the activation of pathways dependent on mitogen-activated protein kinase and nuclear factor-κB (NF-κB; Palumbo et al. 2007, 2009; Penzo et al. 2010), provides a reason for its potent activity as a signal of necrosis (Raucci et al. 2007) triggering inflammation and tissue repair (Sims et al. 2010). HMGB1 acts on stem and precursor cells, recruiting and activating them at sites of damage and injury (Bianchi and Manfredi 2007; Palumbo et al. 2004, 2007, 2009; Limana et al. 2005; Chavakis et al. 2007; Germani et al. 2007; Lolmede et al. 2009).

This action is physiologically important for wound healing and tissue regeneration. Conversely, HMGB1 plays a major role not only in inflammatory and autoimmune diseases, but also in conditions as diverse as cancer and ictogenesis (Mittal et al. 2010; Maroso and Balosso 2010) in which persistent activation of inflammatory and reparative pathways leads to inappropriate tissue remodeling (Vakkila and Lotze 2004; Zeh and Lotze 2005). These studies highlight the potentially noxious outcomes of sustained HMGB1 release in the environment and imply that mechanisms exist that physiologically restrict the biological activity of the molecule. Two such mechanisms involve thrombomodulin (TM) and CD24.

TM is a transmembrane protein that regulates hemostasis through interactions with thrombin; it has been shown to quench HMGB1 inflammatory action by sequestering it via the N-terminal lectin-like domain (Abeyama et al. 2005; Koutsi et al. 2008) and by promoting the proteolytic cleavage of HMGB1 by thrombin (Ito et al. 2008). In vivo, the recombinant human soluble TM reduces HMGB1 levels and increases the survival of rats challenged with LPS (Nagato et al. 2009). The anti-inflammatory activity of the molecule and its ability to reduce HMGB1 levels after LPS challenge have been confirmed by using hTM transgenic mice (Crikis et al. 2010).

HMGB1 also interacts with CD24, a glycosylated glycosyl-phosphatidyl-inositol-anchored membrane protein expressed by immune and stem cells. As a consequence, Siglec 10 is recruited, which contains an immune receptor tyrosine-based inhibitory motif (ITIM); the result is the activation of a negative feedback forward loop, which prevents HMGB1-elicited inflammation by inhibiting NF-kB activation. $CD24^{-/-}$ and siglec $10^{-/-}$ mice are exquisitely sensitive to the systemic effects of endogenous DAMPs, such as HMGB1. In contrast, they are normally resistant to the effects of PAMPs (Bianchi and Manfredi 2009; Chen et al. 2009; Liu et al. 2009). These data hint at an unusual scenario in which HMGB1 differs from PAMPs on the basis that it simultaneously activates inflammation via activatory PRRs (see above) and a CD24/Siglec-10-dependent regulatory pathway. This is possibly advantageous, restraining the ability of HMGB1 to activate inflammation and immunity under conditions of sterile tissue injuries, including vascular diseases (Maugeri et al. 2009) and ischemia/reperfusion (Chavakis et al. 2007).

A failure of these and most likely of other negative feedback regulatory circuits underlie diseases attributable to the deregulated activation of innate immunity. Sepsis is a typical example; it is the leading cause of death in intensive care units in developed high-income countries and represents an urgent and unmet medical need. The first evidence that links HMGB1 to sepsis was obtained more than ten years ago when, in a pioneering study, HMGB1 was identified as a late mediator of lethal systemic inflammation and as being involved in the delayed lethality of endotoxin and systemic inflammation (Wang et al. 1999). Since then, we have gained a better insight into the underling mechanisms, and preclinical studies have validated the possibility of targeting HMGB1 as a therapeutic agent, by using independent approaches (Sims et al. 2010), including anti-HMGB1 antibodies and the A box fragment of HMGB1, which has antagonistic actions. Recently, encouraging results have been obtained, including the blocking of RAGE-HMGB1 signaling (Susa et al. 2009) and, as discussed above, exploiting the regulatory properties of TM (Nagato et al. 2009; Crikis et al. 2010). The identification of HMGB1 polymorphisms as significant factors associated with early and late mortality systemic inflammatory response syndrome and sepsis hints at a possible role for HMGB1 genetics in predictive medicine (Kornblit et al. 2008, 2010).

HMGB1 has also been linked also to tumor formation, progression, and metastasis and to the responses to chemotherapeutics. Its expression is elevated in several solid tumors, and HMGB1 serum levels are often associated with worse prognosis (Sims et al. 2010; Chung et al. 2009; Sparvero et al. 2009). On the other hand, HMGB1 plays a role in the immune responses against tumors elicited by

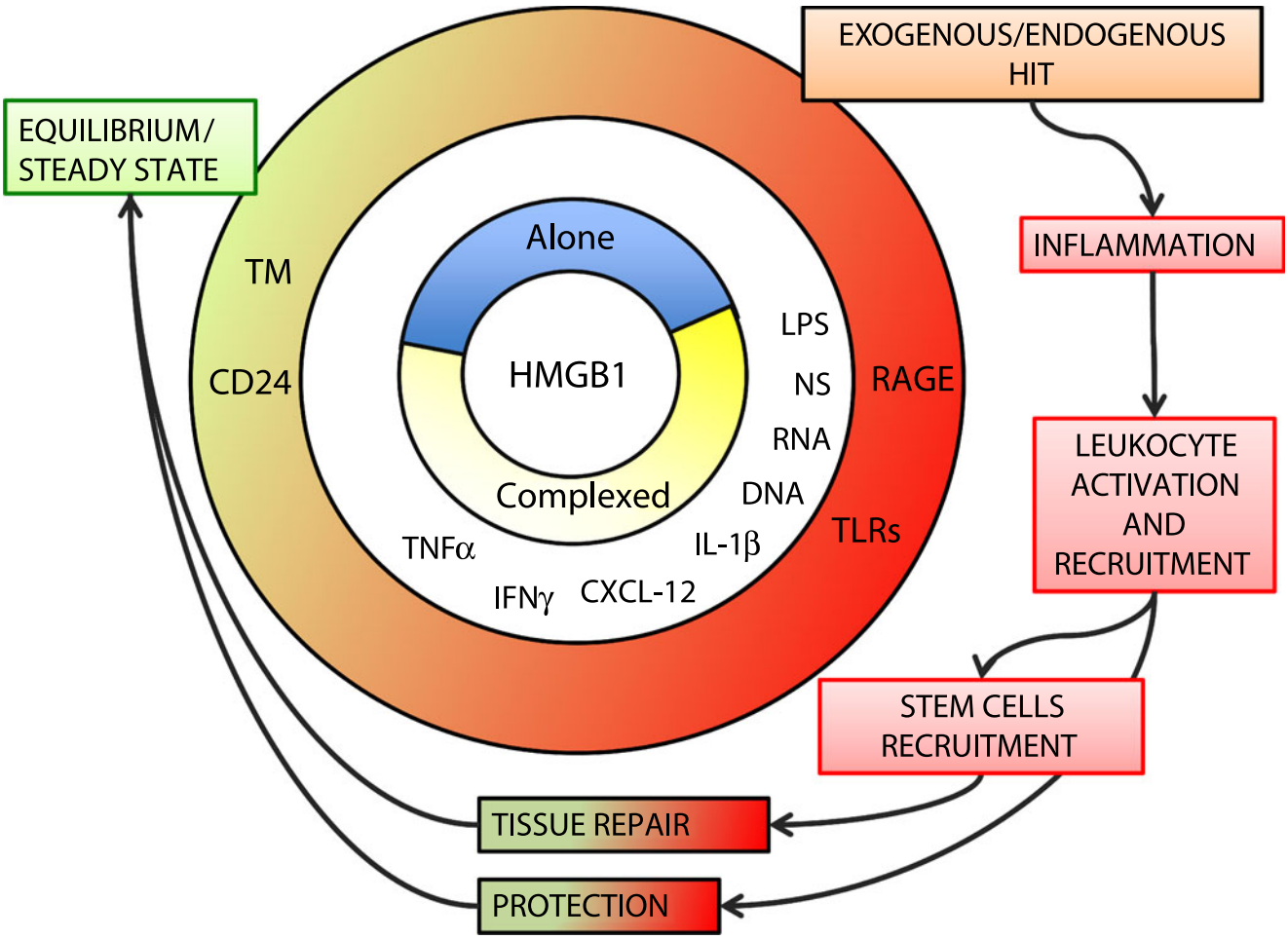

Fig. 2 The many lives of HMGB1: a molecule that shapes inflammation and tissue repair and that depends on environmental conditions and interactions with selected substrates and receptors (*TM* thrombomodulin, *LPS* lipopolysaccharide, *IL-1β* interleukin-1β, *IFNγ* interferon γ, *TNFα* tumor necrosis factor α, *CXCL-12* a lymph-node chemokine, *RAGE* receptor for advanced glycation end-products, *TLRs* Toll-like receptors, *CD24* a glycosylated glycosyl-phosphatidyl-inositol-anchored membrane protein expressed by immune and stem cells, *NS* nucleosomes)

conventional therapies. HMGB1 is released from irradiated and doxorubicin-treated tumor cells, and through TLR4, HMGB1 is efficient in activating DCs to cross-present tumor antigens, suggesting a dual role for the molecule (Apetoh et al. 2007a, b; Campana et al. 2008). The redox state of HMGB1 is important in this context. Reduced HMGB1 binds to RAGE, but not to TLR4, promoting tumor resistance to chemotherapeutic agents such as melphalan, paclitaxel, UV, and oxaliplatin. Oxidized HMGB1, in contrast, apparently increases the cytotoxicity of the agents, with the eventual death of tumor cells (Tang et al. 2010).

Concluding remarks

HMGB1 has many lives (Muller et al. 2001). The concentrated efforts of several groups have revealed some of them, providing hints regarding its multi-layer actions as a master regulator of innate immunity. The studies of the last few years suggest that the possible function of HMGB1 reflects the variable conditions of the extra-cellular environment, by signaling to immune cells the need for an acute and immediate response or for stem cell activation and wound repair, depending on the post-translational modifications of the molecule and/or on the array of substrates with which HMGB1 preferentially interacts. Other DAMPs are possibly more potent at immediately activating the inflammatory response to cell death (Chen et al. 2007; Rock and Kono 2008; Zhang et al. 2010; Manfredi and Rovere-Querini 2010). However, the versatility of HMGB1 makes it an intriguing molecule for unraveling the plasticity of innate immunity; it acts immediately under dangerous conditions and selects, in any given tissue and depending on the nature of injury or of the offending agent, the most appropriate (more effective and less harmful) response to be made.

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