DAMPs, PAMPs and alarmins: all we need to know about danger

Marco E. Bianchi[1]

San Raffaele University, Chromatin Dynamics Unit, Milan, Italy

Abstract: Multicellular animals detect pathogens via a set of receptors that recognize pathogen-associated molecular patterns (PAMPs). However, pathogens are not the only causative agents of tissue and cell damage: trauma is another one. Evidence is accumulating that trauma and its associated tissue damage are recognized at the cell level via receptor-mediated detection of intracellular proteins released by the dead cells. The term "alarmin" is proposed to categorize such endogenous molecules that signal tissue and cell damage. Intriguingly, effector cells of innate and adaptive immunity can secrete alarmins via nonclassical pathways and often do so when they are activated by PAMPs or other alarmins. Endogenous alarmins and exogenous PAMPs therefore convey a similar message and elicit similar responses; they can be considered subgroups of a larger set, the damage-associated molecular patterns (DAMPs). *J. Leukoc. Biol.* 81: 1–5; 2007.

Key Words: *inflammation · immunity · TLR · RAGE*

INTRODUCTION

Multicellular animals must distinguish whether their cells are alive or dead and detect when microorganisms intrude, and have evolved surveillance/defense/repair mechanisms to this end. How these mechanisms are activated and orchestrated is still incompletely understood, and I will argue that that these themes define a unitary field of investigation, of both basic and medical interest.

A complete system for the detection, containment, and repair of damage caused to cells in the organism requires warning signals, cells to respond to them via receptors and signaling pathways, and outputs in the form of physiological responses. Classically, a subset of this system has been recognized and studied in a coherent form: pathogen-associated molecular patterns (PAMPs) are a diverse set of microbial molecules which share a number of different recognizable biochemical features (entire molecules or, more often, part of molecules or polymeric assemblages) that alert the organism to intruding pathogens [1]. Such exogenous PAMPs are recognized by cells of the innate and acquired immunity system, primarily through toll-like receptors (TLRs), which activate several signaling pathways, among which NF-κB is the most distinctive. As a result, some cells are activated to destroy the pathogen and/or pathogen-infected cells, and an immunological response is triggered in order to produce and select specific T cell receptors and antibodies that are best suited to recognize the pathogen on a future occasion. Most of the responses triggered by PAMPs fall into the general categories of inflammation and immunity.

However, pathogens are not the only causative agents of tissue and cell damage: trauma is another one. Tissues can be ripped, squashed, or wounded by mechanical forces, like falling rocks or simply the impact of one's own body hitting the ground. Animals can be wounded by predators. In addition, tissues can be damaged by excessive heat (burns), cold, chemical insults (strong acids or bases, or a number of different cytotoxic poisons), radiation, or the withdrawal of oxygen and/or nutrients. Finally, humans can also be damaged by specially designed drugs, such as chemotherapeutics, that are meant to kill their tumor cells with preference over their healthy cells. Very likely, we would not be here to discuss these issues if evolution had not incorporated in our genetic program ways to deal with these damages, which are not caused by pathogens but are nonetheless real and common enough. Tellingly, inflammation is also activated by these types of insults. A frequently quoted reason for the similarity of the responses evoked by pathogens and trauma is that pathogens can easily breach wounds, and infection often follows trauma; thus, it is generally effective to respond to trauma as if pathogens were present. In my opinion, an additional reason is that pathogens and trauma both cause tissue and cell damage and thus trigger similar responses.

None of these considerations is new; however, a new awareness of the close relationship between trauma- and pathogen-evoked responses emerged from the EMBO Workshop on Innate Danger Signals and HMGB1, which was held in February 2006 in Milano (Italy); many of the findings presented at the meeting are published in this issue of the *Journal of Leukocyte Biology*. At the end of the meeting, Joost Oppenheim proposed the term "alarmin" to differentiate the endogenous molecules that signal tissue and cell damage. Together, alarmins and PAMPs therefore constitute the larger family of damage-associated molecular patterns, or DAMPs.

[1] Correspondence: San Raffaele University, Chromatin Dynamics Unit, via Olgettina 58, 20132 Milan, Italy. E-mail: bianchi.marco@hsr.it

Received March 5, 2006; accepted September 19, 2006.

doi: 10.1189/jlb.0306164

ALARMINS

Alarmins are the equivalent of PAMPs but are endogenous molecules. They have several characteristics: *1)* they are rapidly released following nonprogrammed cell death but are not released by apoptotic cells; *2)* cells of the immune system also can be induced to produce and release alarmins without dying, generally by using specialized secretion systems or by the endoplasmic reticulum (ER)-Golgi secretion pathway; and *3)* they recruit and activate receptor-expressing cells of the innate immune system, including dendritic cells, and thus directly or indirectly also promote adaptive immunity responses. *4)* Finally, alarmins should also restore homeostasis by promoting the reconstruction of the tissue that was destroyed either because of the direct insult or the secondary effects of inflammation. A provisional list of putative alarmins is indicated in **Table 1**; some of these are discussed below.

High mobility group box 1 (HMGB1)

The molecule that fits exemplarily all of the criteria for alarmins is HMGB1.

HMGB1 is a nuclear protein that binds to nucleosomes and promotes DNA bending [2]. It is present at variable levels in most cells [3]. When cells die in a nonprogrammed way, HMGB1 is released in the extracellular medium; in contrast, apoptotic cells modify their chromatin so that HMGB1 binds irreversibly and thus is not released [4].

Myeloid and NK cells, when activated, can secrete their nuclear HMGB1 [5–6; and 7, this issue], without need for further synthesis, after direct translocation to the cytoplasm and accumulation in secretory lysosomes. Neurons, enterocytes, smooth muscle, and endothelial cells can also secrete HMGB1 [8–10], using neither the ER-Golgi nor the secretory lysosome pathways. The development of an ELISPOT assay for HMGB1 [11, this issue] will aid in identifying HMGB1-secreting cell types and stimuli that induce HMGB1 secretion.

The dual origin of extracellular HMGB1 (passively released by necrotic cells and actively secreted from a variety of cells in response to inflammatory stimuli, including the detection of PAMPs) illustrates nicely the convergence of the molecular mechanisms that are brought into action by both infection and trauma. A further turn to this story is that cytolytic cells (antigen-specific CTLs or NK cells) cause the release of HMGB1 from their target cells [12, this issue]. Also extremely intriguing is the recent observation that, although apoptotic cells do not release HMGB1, macrophages engulfing apoptotic cells are induced to secrete HMGB1 [13]. Thus, while the clearance of a few isolated apoptotic cells does not activate inflammation, the clearance of a large number does. Clearly, it appears that immune effector cells can use HMGB1 to mimic trauma in a precisely choreographed way.

HMGB1 has chemotactic activity on monocytes, macrophages, neutrophils and dendritic cells [14; and 15, 16, this issue]. Other cells respond chemotactically to HMGB1, including enterocytes, smooth muscle and endothelial cells [17–19], and HMGB1 has proangiogenic activity [19]. Neurons respond to HMGB1 by extending neurites, a process that also involves cytoskeleton remodeling and has several similarities to chemotaxis [8].

HMGB1 has potent immunostimulatory actions and promotes the maturation of both myeloid and plasmacytoid dendritic cells [20–22].

And finally, HMGB1 can recruit stem cells and promote their proliferation [23]; injection of HMGB1 into the infarcted area of the heart promotes tissue regeneration, and a significant recovery of cardiac performance [24; and 25, this issue). All of these chemotactic and mitogenic activities have been shown or proposed to involve the receptor of advanced glycation endproducts (RAGE), which is a specific HMGB1 receptor expressed at variable level in a variety of cells [26].

An important question is whether HMGB1 can promote directly the secretion of proinflammatory cytokines (TNF, IL-1α/β, Il-6, IL-8) and chemokines (MIP-1α/β) by PBMCs, as initially reported [27]. A direct proinflammatory activity of HMGB1 has not been reproduced consistently (including by my own lab), raising some concern that this might be based on

TABLE 1. A Putative List of Alarmins

Molecule	Passive release[a]	Active nonclassical secretion	Role in inflammation/ immunity	Promoting tissue regeneration
HMGB1	•	•	•	•
S100s		•	•	1
HDGF	•	•		2
HSPs		•	•	
IL-1a		•	•	
Uric acid			•	
Cathelicidins		3	•	•
Defensins		3	•	
EDN		3	•	
Galectins		•	•	
Thymosins			•	•
Nucleolin		•	•	
Annexins		•	•	

A dot indicates that the criterion is fulfilled; absence of a dot means a lack of information. Note that only HMGB1 is known to fulfill all four criteria that I argue can be used to define alarmins. [a] To score positive, the molecules must be released by necrotic cells, but retained by apoptotic cells. 1, S100B is neurotrophic at low concentration but proapoptotic at high concentrations; 2, neurotrophic; and 3, released by neutrophils via degranulation.

the formation of specific complexes with other molecules, for example, single-stranded nucleic acids or LPS [28]. A paper in this issue [29] definitively shows that highly purified recombinant HMGB1 has very weak direct proinflammatory activity; however, it can promote inflammation indirectly, by attracting inflammatory cells. Whether the reported interaction of HMGB1 with TLR receptors [30] depends on its binding to other molecules remains to be established.

S100 proteins

S100 proteins or calgranulins are a group of more than 20 related calcium-binding proteins; in particular, S100A8, S100A9, and S100A12 are expressed by phagocytes and secreted at sites of inflammation [31, this issue]. Like some other alarmins, they lack a leader signal and are secreted via a nonclassical pathway. These proteins induce a specific inflammatory pattern in endothelial cells, with increased vascular permeability and a prothrombotic effect. Moreover, S100B is also released in the brain and has neurotrophic or proapototic effects depending on its concentration [32, this issue].

Interestingly, S100A12 and S100B interact with RAGE (the same receptor of HMGB1), while S100A8/9 may interact with TLR receptors. It is not known whether these molecules could be released by necrotic cells and retained by apoptotic cells.

Hepatoma-derived growth factor

Hepatoma-derived growth factor (HDGF), despite its name, is a protein expressed by neurons. HDGF can be released actively by neurons via a nonclassical pathway and passively by necrotic cells. Most interestingly, HDGF is retained by apoptotic cells. The extracellular protein has neurotrophic properties [33].

Heat shock proteins

Heat shock proteins (HSPs) are a family of proteins that play an essential role as chaperones; they assist the correct folding or refolding of nascent and misfolded proteins. In addition to this intracellular role, they can be secreted actively, again via nonclassical pathways, including exosomes, and released passively by necrotic cells [34, this issue]. Extracellular HSPs can interact with several receptors (including TLRs), inducing the secretion of proinflammatory cytokines. HSPs can also be taken up by antigen-presenting cells, which allows the cross-presentation to the immune system of peptides associated with them.

IL-1α

IL-1α, as its name indicates, is a classical interleukin; its secretion requires processing of the precursor protein and occurs via a nonclassical pathway. However, the precursor of IL-1α translocates into the nucleus of macrophages upon LPS stimulation, and activates transcription by binding to DNA as a transcription factor [35]. Forced expression of the precursor of IL-1α in cells (with concomitant blockage of surface IL-1β receptor (IL-1R) to screen out extracellular effects) either induces the secretion of cytokines or sensitizes cells to respond to subthreshold levels of inflammatory inducers for subsequent cytokine secretion. Thus, IL-1α has both intracellular and extracellular functions, although the former were in this case discovered later.

Uric acid

Shi and Rock recently indentified uric acid as a major alarmin released by injured cells [36]. Interestingly, uric acid is soluble inside cells but precipitates and readily forms monosodium urate (MSU) microcrystals in its extracellular form. Uric acid stimulates dendritic cell maturation and, when coinjected with antigen in vivo, significantly enhances the generation of responses from CD8+ T cells. Eliminating uric acid in vivo inhibits the immune response to antigens associated with transplanted syngeneic cells and the proliferation of autoreactive T cells in a transgenic diabetes model. In contrast, uric acid depletion does not reduce the stimulation of T cells by mature, activated antigen-presenting cells [37].

Extracellular uric acid also has major inflammatory properties, most evident when it accumulates in tissues and causes gout. Interestingly, MSU crystals engage the inflammasome, resulting in the production of active IL-1β and IL-18. Macrophages from mice deficient in IL-1R or in various components of the inflammasome, such as caspase-1, ASC, and NALP3, are defective in MSU-induced cytokine secretion and have reduced inflammation [38].

ALARMIN RECEPTORS AND SIGNAL TRANSDUCTION PATHWAYS

As already indicated in the preceding section, some alarmins can engage TLRs or IL-1R, which are classical receptors leading to inflammatory and immune responses. RAGE is another receptor that appears to play a key role in alarmin function. RAGE is a multiligand receptor binding advanced glycation end products (AGEs), some S100s, amyloid peptide and HMGB1 [39]. RAGE knockout mice are viable and fertile but display a wide range of defects. Most of these defects are subtler than expected, leading to the suggestion that other receptors with overlapping function might exist. Syndecan has been identified as another HMGB1 receptor [40].

Interestingly, TLRs, IL-1R, and RAGE engagement all lead to NF-κB activation, suggesting that both receptor usage and signaling pathways evoke similar responses when cells are activated by PAMPs and alarmins. Moreover, PAMPs and alarmins might synergistically reinforce each other both at the receptor level and in the activation of transcriptional responses. An interesting example, whereby HMGB1 appears to sensitize dentritic cells by increasing TLR4 expression, is shown in this issue [41].

Since they have potent extracellular functions, alarmins have to be kept in check and counteracted by inhibiting molecules. Extracellular uric acid is eliminated by uricase. HMGB1-caused inflammation can be dampened by soluble RAGE and by thrombomodulin, a cell surface protein of endothelial cells that binds and activates thrombin [42]. Anti-HMGB1-neutralizing antibodies are found in a fraction of human subjects, even without overt clinical symptoms [43, this

issue] and may modulate the responses to extracellular HMGB1.

ALARMIN PATHOLOGIES

Extranuclear expression of HMGB1 has been involved in a number of pathogenic conditions: sepsis [44], arthritis [45, 46], atherosclerosis [10], systemic lupus erythematosus (SLE) [47], cancer [48] and hepatitis [49, this issue]. Uric acid has been known to be the aethiologic agent for gout since the 19th century. S100s may be involved in arthritis [31, this issue] and psoriasis [50]. However, although it is clear that excessive alarmin expression might lead to acute and chronic diseases, the molecular mechanisms underlying these effects are still largely unexplored.

CONCLUSION

The short list of alarmins presented above is certainly both provisional and incomplete and serves only as an introduction to the alarmin concept and to the papers published in this issue of JLB. Other molecules may be added to the list, including cathelicidins, defensins and eosinophil-derived neurotoxin (EDN) [51], galectins [52], thymosins [53], nucleolin [54], and annexins [55; and 56, this issue]; more will emerge with time. Eventually, the concept will have to be revised and adjusted to the growing information. Indeed, I have previously argued that any misplaced protein in the cell can signal damage [57], and Polly Matzinger has proposed that any hydrophobic surface ("Hyppo", or Hydrophobic protein part) might act as a DAMP [58].

As most concepts in biology, the alarmin category serves for our understanding and does not correspond to a blueprint or a plan in the construction of organisms. Biology proceeds via evolution, and evolution is a tinkerer or bricoleur, finding new functions for old molecules. In this, the reuse of cellular components as signals for alerting cells to respond to damage and danger, is a prime example.

ACKNOWLEDGMENTS

I thank all participants to the EMBO Workshop on Innate Danger Signals and HMGB1 for information and inspiration. Work in my lab is supported by the Italian Association for Cancer Research (AIRC), the Association for International Cancer Research (AICR), the Ministry of University and Research (MUR), Fondazione Cariplo and Fondazione Monte dei Paschi di Siena (FMPS). The author declares no direct financial interest in this study; however, he is part-owner of HMG-Biotech, a biotech company which sells HMGB1-related goods and services.

REFERENCES

1. Janeway, C. A., Jr., Medzhitov, R. (2002) Innate immune recognition. *Annu. Rev. Immunol.* **20,** 197–216.
2. Agresti, A., Bianchi, M. E. (2003) HMGB proteins and gene expression. *Curr. Opin. Genet. Dev.* **13,** 170–178.
3. Müller, S., Ronfani, L., Bianchi, M. E. (2004) Regulated expression and subcellular localization of HMGB1, a chromatin protein with a cytokine function. *J. Intern. Med.* **255,** 332–343.
4. Scaffidi, P., Misteli, T., Bianchi, M. E. (2002) Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* **418,** 191–195.
5. Gardella, S., Andrei, C., Ferrera, D., Lotti, L. V., Torrisi, M. R., Bianchi, M. E., Rubartelli, A. (2002) The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. *EMBO Rep.* **3,** 995–1001.
6. Bonaldi, T., Talamo, F., Scaffidi, P., Ferrera, D., Porto, A., Bachi, A., Rubartelli, A., Agresti, A., Bianchi, M. E. (2003) Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. *EMBO J.* **22,** 5551–5560.
7. Semino, C., Ceccarelli, J., Lotti, L.V., Torrisi, M.R., Angelini, G., Rubartelli, A. (2007) The maturation potential of NK cell clones toward autologous dendritic cells correlates with HMGB1 secretion. *J. Leukoc. Biol.* **81,** 92–99.
8. Rauvala, H., Merenmeri, J., Pihlaskari, R., Korkolainen, M., Huhtala, M.-L., Panula, P. (1988) The adhesive and neurite-promoting molecule p30: analysis of the amino-terminal sequence and production of antipeptide antibodies that detect p30 at the surface of neuroblastoma cells and of brain neurons. *J. Cell Biol.* **107,** 2293–2305.
9. Liu, S., Stolz, D.B., Sappington, P.L., Macias, C.A., Killeen, M.E., Tenhunen, J.J., Delude, R.L., Fink, M.P. (2006) HMGB1 is secreted by immunostimulated enterocytes and contributes to cytomix-induced hyperpermeability of Caco-2 monolayers. *Am. J. Physiol. Cell Physiol.* **290,** C990-9.
10. Porto, A., Palumbo, R., Pieroni, M., Aprigliano, G., Chiesa, R., Sanvito, F., Maseri, A., Bianchi, M. E. (2006) Smooth muscle cells in human atherosclerotic plaques secrete and proliferate in response to High Mobility Protein Box 1. *FASEB J.*, **20**, E1–E9.
11. Wahamaa, H., Vallerskog, T., Qin, S., Lunderius, C., Larosa, G., Andersson, U., Erlandsson-Harris, H. (2007) HMGB1-secreting capacity of multiple cell lineages revealed by a novel HMGB1 ELISPOT assay. *J. Leukoc. Biol.* **81,** 129–136.
12. Ito, N., Demarco, R.A., Mailliard, R.B., Han, J., Rabinowich, H., Kalinski, P., Stolz, D.B., Zeh, H.J., 3rd, Lotze, M.T. (2007) Cytolytic cells induce HMGB1 release from melanoma cell lines. *J. Leukoc. Biol.* **81,** 75–83.
13. Qin, S., Wang, H., Yuan, R., Li, H., Ochani, M., Ochani, K., Rosas-Ballina, M., Czura, C. J., Huston, J. M., Miller, E., et al. (2006) Role of HMGB1 in apoptosis-mediated sepsis lethality. *J. Exp. Med.* **203,** 1637–1642.
14. Dumitriu, I. E., Baruah, P., Manfredi, A. A., Bianchi, M. E., Rovere-Querini, P. (2005) HMGB1: guiding immunity from within. *Trends Immunol.* **26,** 381–387.
15. Yang, D., Chen, Q., Yang, H., Tracey, K.J., Bustin, M., Oppenheim, J.J. (2006) High mobility group box-1 (HMGB1) protein induces the migration and activation of human dendritic cells and acts as an alarmin. *J. Leukoc. Biol.* **81,** 59–66.
16. Dumitriu, I.E., Bianchi, M.E., Bacci, M., Manfredi, A.A., Rovere-Querini, P. (2007) The secretion of HMGB1 is required for the migration of maturing dendritic cells. *J. Leuk. Biol.* **81,** 84–91.
17. Degryse, B., Bonaldi, T., Scaffidi, P., Muller, S., Resnati, M., Sanvito, F., Arrigoni, G., Bianchi, M. E. (2001) The high mobility group (HMG) boxes of the nuclear protein HMG1 induce chemotaxis and cytoskeleton reorganization in rat smooth muscle cells. *J. Cell Biol.* **152,** 1197–1206.
18. Sappington, P. L., Yang, R., Yang, H., Tracey, K. J., Delude, R. L., Fink, M. P. (2002) HMGB1 B box increases the permeability of Caco-2 enterocytic monolayers and impairs intestinal barrier function in mice. *Gastroenterology* **123,** 790–802.
19. Mitola, S., Belleri, M., Urbinati, C., Coltrini, D., Sparatore, B., Pedrazzi, M., Melloni, E., Presta, M. (2006) Cutting edge: extracellular high mobility group box-1 protein is a proangiogenic cytokine. *J. Immunol.* **176,** 12–15.
20. Dumitriu, I. E., Baruah, P., Bianchi, M. E., Manfredi, A. A., Rovere-Querini, P. (2005) Requirement of HMGB1 and RAGE for the maturation of human plasmacytoid dendritic cells. *Eur. J. Immunol.* **35,** 2184–2190.
21. Rovere-Querini, P., Capobianco, A., Scaffidi, P., Valentinis, B., Catalanotti, F., Giazzon, M., Dumitriu, I. E., Muller, S., Iannacone, M., Traversari, C., et al. (2004) HMGB1 is an endogenous immune adjuvant released by necrotic cells. *EMBO Rep.* **5,** 825–830.
22. Messmer, D., Yang, H., Telusma, G., Knoll, F., Li, J., Messmer, B., Tracey, K. J., Chiorazzi, N. (2004) High mobility group box protein 1: an endogenous signal for dendritic cell maturation and Th1 polarization. *J. Immunol.* **173,** 307–313.

23. Palumbo, R., Sampaolesi, M., De Marchis, F., Tonlorenzi, R., Colombetti, S., Mondino, A., Cossu, G., Bianchi, M. E. (2004) Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. *J. Cell Biol.* **164,** 441–449.
24. Limana, F., Germani, A., Zacheo, A., Kajstura, J., Di Carlo, A., Borsellino, G., Leoni, O., Palumbo, R., Battistini, L., Rastaldo, R., et al. (2005) Exogenous high-mobility group box 1 protein induces myocardial regeneration after infarction via enhanced cardiac C-kit+ cell proliferation and differentiation. *Circ. Res.* **97,** e73–e83.
25. Germani, A., Limana, F., Capogrossi, M.C. (2006) Pivotal Advance: High-mobility group box 1 protein—a cytokine with a role in cardiac repair. *J. Leukoc. Biol.* **81**: 41–45.
26. Hori, O., Yan, S. D., Ogawa, S., Kuwabara, K., Matsumoto, M., Stern, D., Schmidt, A. M. (1995) The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. *J. Biol. Chem.* **270,** 25752–25761.
27. Andersson, U., Wang, H., Palmblad, K., Aveberger, A. C., Bloom, O., Erlandsson-Harris, H., Janson, A., Kokkola, R., Zhang, M., Yang, H., et al. (2000) High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. *J. Exp. Med.* **192,** 565–570.
28. Erlandsson-Harris, H., Raucci, A. (2006) Alarmin(g) news about danger. Workshop on Innate Danger Signals and HMGB1. *EMBO Rep.* **7,** 774–778.
29. Rouhiainen, A., Tumova, S., Valmu, L., Kalkkinen, N., Rauvala, H. (2006) Analysis of proinflammatory activity of highly purified eukaryotic recombinant HMGB1 (Amphoterin). *J. Leukoc. Biol.* **81,** 49–58.
30. Park, J. S., Svetkauskaite, D., He, Q., Kim, J. Y., Strassheim, D., Ishizaka, A., Abraham, E. (2004) Involvement of TLR2 and TLR4 in cellular activation by high mobility group box 1 protein (HMGB1). *J. Biol. Chem.* **279,** 7370–7377.
31. Foell, D., H., W., Vogl, T., Roth, J. (2007) S100 proteins expressed in phagocytes: a novel group of Damage Associated Molecular Pattern molecules. *J. Leukoc. Biol.* **81**: 28–37.
32. Bianchi, R., Adami, C., Giambanco, I., Donato, R. (2006) S100B binding to RAGE in microglia stimulates COX-2 expression. *J. Leukoc. Biol.* **81,** 108–118.
33. Zhou, Z., Yamamoto, Y., Sugai, F., Yoshida, K., Kishima, Y., Sumi, H., Nakamura, H., Sakoda, S. (2004) Hepatoma-derived growth factor is a neurotrophic factor harbored in the nucleus. *J. Biol. Chem.* **279,** 27320–27326.
34. Schmitt, E., Gehrmann, M., Brunet, M., Multhoff, G., Garrido, C. (2007) Intracellular and extracellular functions of Heat Shock Proteins: Repercussions in cancer therapy. *J. Leukoc. Biol.* **81,** 15–27.
35. Werman, A., Werman-Venkert, R., White, R., Lee, J. K., Werman, B., Krelin, Y., Voronov, E., Dinarello, C. A., Apte, R. N. (2004) The precursor form of IL-1α is an intracrine proinflammatory activator of transcription. *Proc. Natl. Acad. Sci. USA* **101,** 2434–2439.
36. Shi, Y., Evans, J. E., Rock, K. L. (2003) Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* **425,** 516–521.
37. Shi, Y., Galusha, S. A., Rock, K. L. (2006) Cutting edge: elimination of an endogenous adjuvant reduces the activation of CD8 T lymphocytes to transplanted cells and in an autoimmune diabetes model. *J. Immunol.* **176,** 3905–3908.
38. Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., Tschopp, J. (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* **440,** 237–241.
39. Bierhaus, A., Humpert, P. M., Morcos, M., Wendt, T., Chavakis, T., Arnold, B., Stern, D. M., Nawroth, P. P. (2005) Understanding RAGE, the receptor for advanced glycation end products. *J. Mol. Med.* **83,** 876–886.
40. Salmivirta, M., Rauvala, H., Elenius, K., Jalkanen, M. (1992) Neurite growth-promoting protein (amphoterin, p30) binds syndecan. *Exp. Cell Res.* **200,** 444–451.
41. Tsung, A., Zheng, N., Jeyabalan, G., Izuishi, K., R., K.J., Geller, D.A., Lotze, M.T., Lu, L., Billiar, T.R. (2007) Increasing numbers of hepatic dendritic cells promote HMGB1 mediated ischemia-reperfusion injury. *J. Leukoc. Biol.* **81,** 119–128.
42. Abeyama, K., Stern, D. M., Ito, Y., Kawahara, K. I., Yoshimoto, Y., Tanaka, M., Uchimura, T., Ida, N., Yamazaki, Y., Yamada, S., et al. (2005) The N-terminal domain of thrombomodulin sequesters high-mobility group-B1 protein, a novel antiinflammatory mechanism. *J. Clin. Invest.* **115,** 1267–1274.
43. Urbonaviciute, V., Fürnrohr, B.G., Weber, C., Haslbeck, M., Wilhelm, S., Herrmann, M., Voll, R.E. (2007) Factors masking HMGB1 in human serum and plasma. *J. Leukoc. Biol.* **81,** 67–74.
44. Yang, H., Ochani, M., Li, J., Qiang, X., Tanovic, M., Harris, H. E., Susarla, S. M., Ulloa, L., Wang, H., DiRaimo, R., et al. (2004) Reversing established sepsis with antagonists of endogenous high-mobility group box 1. *Proc. Natl. Acad. Sci. USA* **101,** 296–301.
45. Taniguchi, N., Kawahara, K., Yone, K., Hashiguchi, T., Yamakuchi, M., Goto, M., Inoue, K., Yamada, S., Ijiri, K., Matsunaga, S., et al. (2003) High mobility group box chromosomal protein 1 plays a role in the pathogenesis of rheumatoid arthritis as a novel cytokine. *Arthritis Rheum.* **48,** 971–981.
46. Kokkola, R., Li, J., Sundberg, E., Aveberger, A. C., Palmblad, K., Yang, H., Tracey, K. J., Andersson, U., Harris, H. E. (2003) Successful treatment of collagen-induced arthritis in mice and rats by targeting extracellular high mobility group box chromosomal protein 1 activity. *Arthritis Rheum.* **48,** 2052–2058.
47. Popovic, K., Ek, M., Espinosa, A., Padyukov, L., Harris, H. E., Wahren-Herlenius, M., Nyberg, F. (2005) Increased expression of the novel proinflammatory cytokine high mobility group box chromosomal protein 1 in skin lesions of patients with lupus erythematosus. *Arthritis Rheum.* **52,** 3639–3645.
48. Taguchi, A., Blood, D. C., del Toro, G., Canet, A., Lee, D. C., Qu, W., Tanji, N., Lu, Y., Lalla, E., Fu, C., et al. (2000) Blockage of RAGE-amphoterin signalling suppresses tumour growth and metastasis. *Nature* **405,** 354–360.
49. Sitia, G., Iannacone, M., Müller, S., Bianchi, M.E., Guidotti, L.G. (2007) Treatment with HMGB1 inhibitors diminishes CTL-induced liver disease in HBV transgenic mice. *J. Leukoc. Biol.* **81,** 100–107.
50. Zenz, R., Eferl, R., Kenner, L., Florin, L., Hummerich, L., Mehic, D., Scheuch, H., Angel, P., Tschachler, E., Wagner, E. F. (2005) Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. *Nature* **437,** 369–375.
51. Oppenheim, J. J., Yang, D. (2005) Alarmins: chemotactic activators of immune responses. *Curr. Opin. Immunol.* **17,** 359–365.
52. Liu, F. T., Rabinovich, G. A. (2005) Galectins as modulators of tumour progression. *Nat. Rev. Cancer* **5,** 29–41.
53. Goldstein, A. L., Hannappel, E., Kleinman, H. K. (2005) Thymosin beta4: actin-sequestering protein moonlights to repair injured tissues. *Trends Mol. Med.* **11,** 421–429.
54. Christian, S., Pilch, J., Akerman, M. E., Porkka, K., Laakkonen, P., Ruoslahti, E. (2003) Nucleolin expressed at the cell surface is a marker of endothelial cells in angiogenic blood vessels. *J. Cell Biol.* **163,** 871–878.
55. Gerke, V., Creutz, C. E., Moss, S. E. (2005) Annexins: linking Ca^{2+} signalling to membrane dynamics. *Nat. Rev. Mol. Cell Biol.* **6,** 449–461.
56. Munoz, L.E., Franz, S., Pausch, F., Furnrohr, B., Sheriff, A., Vogt, B., Kern, P.M., Baum, W., Stach, C., von Laer, D., Brachvogel, B., Poschl, E., Herrmann, M., Gaipl, U.S. (2006) The influence on the immunomodulatory effects of dying and dead cells of Annexin V. *J. Leukoc. Biol.* **81,** 6–14.
57. Bianchi, M. E. (2004) Significant (re)location: how to use chromatin and/or abundant proteins as messages of life and death. *Trends Cell Biol.* **14,** 287–293.
58. Seong, S. Y., Matzinger, P. (2004) Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat. Rev. Immunol.* **4,** 469–478.