High mobility group box-1 recognition: The beginning of a RAGEless era?

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High mobility group box 1 (HMGB1) is a molecular alarm signal that triggers an immune response when released. It was assumed that the receptor for advanced glycation end-products (RAGE) would mediate the signal to the immune system. Recently, pattern recognition receptors that are triggered by molecules of bacterial origin (the Toll-like receptor (TLR) family) were shown to also respond to HMGB1. Now two papers establish the TLR4–HMGB1 axis as proinflammatory, eventually leading to disparate conditions like seizures or skin cancer. These reports add a new twist to our understanding of the mode of action of the alarm signal HMGB1.

High mobility group box 1 (HMGB1) is a jack of all trades. Apart from being a non-histone chromatin associated protein and a regulator of gene function, it is also increasingly clear that it acts as an alarmin. Alarmins are endogenous substances that alert the body's immune system of the presence of danger, such as necrotic cell death, hypoxia or severe injury. The immune system evolved to recognize the presence of these alarmins, as sites of necrosis or injury are also frequent portals of entry for pathogens. Indeed, receptors that recognize bacteria and other pathogens such as the toll-like and the NOD-like receptor families (TLR and NLR, respectively) also recognize alarmins. The most prototypical of the TLR receptors is TLR4, the receptor for lipopolysaccharide (LPS), a component of the Gram negative bacterial cell wall. Two recent papers, Mittal et al (2010) and Maroso et al (2010) provide clear evidence that the recognition of HMGB1 by TLR4 is at the heart of understanding the inflammatory basis of two very common disease processes, skin cancer and epilepsy.

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Further complicating our understanding of the in vivo biology of this pathway is the fact that HMGB1 signals via many different receptors. The unusual versatility and complex biological behaviour of this protein can be explained at multiple levels:

First, HMGB1 appears to be subject to a large number of post-translational modifications, including acetylation, phosphorylation, methylation and/or oxidation. These modifications can regulate HMGB1 trafficking from the cytoplasm to the nucleus, its retention by apoptotic DNA, or its active release by inflammatory cells. Our understanding of how these modifications alter HMGB1 receptor preference is fragmentary at best. More is known about their biological relevance. For example, oxidized HMGB1, which is released from apoptotic...
cells, induces tolerance to dead cells by promoting their phagocytosis.

Secondly, consistent with its function as a DNA binding protein, extracellular HMGB1 has a natural propensity to bind to negatively charged molecules such as DNA, RNA, nucleosomes and pathogen associated molecules like LPS (an endotoxin) or immune activating cytokines like interleukin-1 (IL-1) (Bianchi, 2009). The binding of HMGB1 to LPS and IL-1 does not neutralize them, but rather leads to the formation of an activated complex that triggers the respective TLR or cytokine receptors better. Therefore, any demonstration of HMGB1 directly triggering a particular receptor should be interpreted in the context of this complex formation and carries an inherent risk that co-purified or complexed TLR agonist might be the real trigger of immune activation. Taking this into account, the array of receptors that respond to HMGB1 (such as the cell-membrane expressed TLR2 and TLR4 and the endosomal TLR3, TLR7 or TLR9) is ever expanding. DNA bound to HMGB1 is a clear sign of necrotic cell death, and this complex triggers TLR9 receptor signalling in conjunction with RAGE. Nucleosome bound HMGB1 (occurring as a result of incomplete apoptotic processing or secondary necrosis of apoptotic cells) will also activate antigen presenting cells through triggering of the TLR2 receptor. The fact that ultra-purified HMGB1 does not induce immune responses (Rouhiainen et al, 2007) indicates that complexes of classical TLR agonists with HMGB1 are important in sending alarm messages.

At first sight this complex signalling cross-talk may appear to be little more than molecular chatter. However, a closer look points to a selective propagation of information. It is in that light that we see evidence of TLR4 emerging as a transmitter of the HMGB1 message, as shown by Mittal et al (2010) and Maroso et al (2010). It has previously been described that HMGB1 excerpts biological effects via triggering of the TLR4 receptor, but until recently there was lack of evidence to support the idea of a critical role of HMGB1 induced TLR4 activation in disease pathogenesis. The TLR4 signalling cascade has been studied in great detail and involves activation via the adaptor molecules Myd88 and TRIF. MyD88 is at the crossroads of many signalling pathways involving TLR receptors and the IL1R, and a common end result is NF-κB driven induction of inflammatory cytokines and chemokines, resulting in inflammation. Note that MyD88 has not only been implicated as a key player in defence mechanisms against microbial infection and inflammation, but also in several mouse tumourigenesis models.

Mittal et al (2010) employed a number of mouse models to elucidate the contributions of different TLRs to tumor initiation and progression using a dual stage tumour induction protocol [croton oil (CO)-induced inflammation and sub-

Croton oil applied to the skin causes cell death that is at the origin of inflammation. According to Mittal et al, HMGB1 released from dying cells will activate TLR4 in skin cells, leading to inflammation (this process is analogous to inflammation induced in nervous tissue by kainate-induced overstimulated neurons, as shown by Maroso et al (2010)). The presence of TLR4 is necessary in both radio-resistant and Bone marrow derived cells. Aided by several chemokines, HMGB1 signalling via TLR4 in inflammatory cells activates immune responses leading to skin cancer (left). However, what remains to be explained is whether HMGB1 will act alone or if binding to an agonist (like LPS or IL-1) is needed in vivo to efficiently activate TLR4 (middle). Furthermore, the effect of HMGB1 on other endogenous receptors (especially RAGE) has not been ruled out. As evidence builds up for a role of RAGE in multiple types of cancer or other immune conditions, RAGE activation might be necessary for efficient HMGB1–TLR4 signalling (right). Because there are many other alarm signals that are released in similar conditions (like, e.g. ATP or uric acid), a reasonable assumption is that they might also play a role in the onset of inflammation.
sequent progression to skin tumours] (Fig 1). They showed that mice deficient for TLR4 or MyD88 are less prone to skin tumor progression. Strikingly, skin inflammation could be induced by CO in a sterile environment, as removal of the skin microbiota did not reduce inflammation, nor did administration of the TLR4 agonist LPS induce skin inflammation. Rather, CO application induced necrotic cell death, cytoplasmic relocation and release of HMGB1, thereby triggering the TLR4 pathway. In support of this, in vivo neutralization of HMGB1 using the BoxA domain of the protein reduced CO-induced skin inflammation. Mice lacking TLR2 or TLR9 (which can also respond to HMGB1) showed no apparent protection against the development of skin tumours. Strikingly, TLR4 triggering of both hematopoietic and stromal cells was necessary for tumour-triggering of both hematopoietic and stromal cells was necessary for tumour-progression to skin tumours. Strikingly, TLR4 agonist LPS induces skin inflammation. Rather, CO application induced necrotic cell death, cytoplasmic relocation and release of HMGB1, thereby triggering the TLR4 pathway. In support of this, in vivo neutralization of HMGB1 using the BoxA domain of the protein reduced CO-induced skin inflammation. Mice lacking TLR2 or TLR9 (which can also respond to HMGB1) showed no apparent protection against the development of skin tumours. Strikingly, TLR4 triggering of both hematopoietic and stromal cells was necessary for tumourogenesis and inflammation. Somewhat surprisingly however, rage−/− mice were not used in this study, leaving open the possibility that TLR4 works together with RAGE to induce this type of response.

Like skin cancer, epilepsy is associated with inflammation. Maroso et al (2010) used several mouse models to elucidate the effect of alarmins and sterile inflammation on the initiation and progression of seizures in models of epilepsy. Through use of the pharmacological compounds that trigger the N-methyl-D-aspartate (glutamate) receptor, they demonstrated that HMGB1 is an ictogenic (i.e. a proconvulsant) mediator, as both cytoplasmic and extracellular HMGB1 increased in affected tissues. TLR4 deficient mice or mice treated with BoxA domain peptide were shown to be impervious to kainate as a seizure inducer. This pathway might be therapeutically amenable, as pharmacological TLR4 antagonists also reduced seizure activity and HMGB1 and TLR4 were found upregulated in patients with temporal lobe epilepsy, a particularly severe and therapy resistant form of the disease. Again rage−/− mice were not used in these model systems, leaving open the possibility that HMGB1 triggers TLR4 in combination with RAGE triggering.

These two papers bring the HMGB1–TLR4 pathway into the spotlight by showing its direct involvement in both seizures and cancer, as a result of their role in promoting inflammation induced by sterile cell death and/or cellular stress. For both papers it is the first time that the effect of TLR4 activation via HMGB1 is shown to be the trigger for inflammation. Despite the exciting nature of these findings, many questions remain open. Because of the generally accepted view of TLR4 as a receptor for LPS, Mittal et al (2010) tested the effect of LPS on their seizure induction protocol. Contradictory to what might be expected, LPS reduces the threshold for seizures but offers protection against skin cancer. Interestingly, both overstimulated neurons and injured skin cells produce and release higher levels of HMGB1 in vitro, although no in vivo data are reported. One possibility that remains is that the association between HMGB1 or TLR4 (or other receptors) might be dependent on the extracellular concentrations of HMGB1. In this context, HMGB1 might be a preferential activator of RAGE, also promoting stimulation of other receptors when present in low concentrations (or the other way around).

Care has to be taken in interpreting the results, as HMGB1 administered to the same C3H/HeJ mice, which lack a functional TLR4, induces lethality, which points to a TLR4-independent pathway for HMGB1 (Sims et al, 2010). It also remains unclear whether HMGB1 is modified or binds to other partners in vivo.

Is it the beginning of a RAGEless era? It is too early to say. Indeed, Sims et al (2010) recently argued a predominant role for the HMGB1–RAGE axis in the development of inflammation and cancer. The beauty of biological systems is their complexity. Nevertheless, it is clear that a more comprehensive understanding of the role of HMGB1 and its receptors in complex disorders like sepsis, diabetes, cancer, neurodegeneration or autoimmune disease could provide new therapeutic avenues.

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