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Stress sounds the alarmin: the role of the danger-associated molecular pattern HMGB1 in stress-induced neuroinflammatory priming

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Abstract

High mobility group box-1 (HMGB1) is an endogenous danger signal or alarmin that mediates activation of the innate immune response including chemotaxis and pro-inflammatory cytokine release. HMGB1 has been implicated in the pathophysiology of several neuroinflammatory conditions including ischemia, traumatic brain injury, seizure and chronic ethanol use. In the present review, the unique structural and functional properties of HMGB1 will be explored including its affinity for multiple pattern recognition receptors (TLR2/TLR4), redox sensitivity and adjuvant-like properties. In light of recent evidence suggesting that HMGB1 may also mediate stress-induced sensitization of neuroinflammatory responses, mechanisms of HMGB1 action in neuroinflammatory priming are explored. A model of neuroinflammatory priming is developed wherein glucocorticoids induce synthesis and release of HMGB1 from microglia, which signals through TLR2/TLR4, thereby priming the NLRP3 inflammasome. We propose that if GCs reach a critical threshold as during a fight/flight response, they may thus function as an alarmin by inducing HMGB1, thereby preparing an organism's innate immune system (NLRP3 inflammasome priming) for subsequent immune challenges such as injury, trauma or infection, which are more likely to occur during a fight/flight response. In doing so, GCs may confer a significant survival advantage by enhancing the central innate immune and sickness response to immune challenges.

Keywords

microglia; DAMP; HMGB1; neuroinflammation; priming; glucocorticoid; stress

1. Introduction

First proposed by Matzinger in 1994, the danger model of immunogenicity postulated that the immune system generates a response to an exogenous or endogenous immunogenic

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stimulus only if that stimulus induces cellular damage or distress and the consequent release of danger signals (Matzinger, 1994). That is, the danger model proposes that the salient immunological feature of a stimulus is not its “foreignness” (self/non-self model of immunogenicity), but rather its capacity to induce tissue stress or destruction (see review by Pradeau and Cooper comparing the danger model and self/non-self models (Pradeu and Cooper, 2012)). Thus, the immune system will respond to a stimulus only if that stimulus results in the release of endogenous danger-associated molecular patterns (DAMPs), also known as alarmins, which signal cellular damage and activate the innate immune system (Bianchi, 2007). The explanatory power of the danger model is particularly relevant to pathophysiological conditions involving sterile injury or trauma. Under such conditions, an inflammatory event is induced in the absence of infection or exposure to pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), which are considered exogenous DAMPs.

Inflammation in the absence of stimulation by PAMPs is not restricted to the periphery. Neuroinflammation and the behavioral sequelae of neuroinflammation (sickness behavior) can be induced under sterile conditions within the CNS such as ischemia, seizure or even psychological stress. Moreover, exposure to exogenous PAMPs such as LPS in the periphery induces neuroinflammatory processes without entering the CNS. Clearly then, peripheral immune-to-brain signaling pathways can initiate neuroinflammation after peripheral exposure to exogenous PAMPs. These pathways have been well characterized (Maier and Watkins, 2003), but the proximate mediator(s) released within the CNS that directly signals innate immune cells (e.g. microglia) to induce a pro-inflammatory response whether in the context of an exogenous PAMP (e.g. peripheral LPS exposure) or endogenous (sterile injury) immunological threat remains unknown. Although the danger model was developed to help understand peripheral innate immunity, extension to the CNS raises the intriguing possibility that DAMPs released within the brain in response to exogenous or endogenous immunogenic stimuli may play a pivotal role as proximate mediators of neuroinflammatory processes. In the present review, we will explore and develop this notion with a focus on the endogenous DAMP, high mobility group box-1 (HMGB1), which is considered an archetypal alarmin (Bianchi, 2009) as well as a “master regulator” of innate immunity (Castiglioni et al., 2011). While many endogenous DAMPs have been identified (e.g. heat shock proteins, HSPs; uric acid; S100 proteins)(Bianchi, 2007), the scope of the present review will be restricted to examining HMGB1 as it has been implicated in several neuroinflammatory conditions, including stress-induced pro-inflammatory responses, and has unique structural and functional properties, which form the basis of its pleiotropic effects on innate immune cells.

2. HMGB1

HMGB1 shares several molecular characteristics with other DAMPs (e.g. HSPs, uric acid or S100 proteins) including the capacity to elicit a pro-inflammatory response predominately through Toll-like receptors (TLRs). However, HMGB1 exhibits several molecular features, which clearly distinguish it from other alarmins. As developed below, these features include a redox-sensitive capacity to rapidly shift between functional states as well as a capacity to bind and amplify the pro-inflammatory effects of cytokines and PAMPs.

2.1. Primary Structure and Function

HMGB1 is a 25 kDa protein consisting of a 216 amino acid sequence, which is highly conserved across species. The primary structure of HMGB1 is comprised of two DNA binding domains, an A box and B box, and a negatively charged acidic tail (C-terminus) (Yang and Tracey, 2005). The B box domain contains the cytokine-inducing region of HMGB1 (specifically cysteine 106; see below), whereas the A box binds to receptor targets, but does not initiate receptor signaling (Li et al., 2003). Interestingly, the A box fragment functions to competitively antagonize the pro-inflammatory effects of HMGB1 since it binds to receptors without initiating signaling (Yang et al., 2004). It should be noted that the A Box fragment has not been detected *in vivo*, and so it is unclear whether the antagonistic properties of the A box domain play a physiological role in the regulation of HMGB1 signaling. HMGB1 is primarily located in the nucleus of most cells (Yang et al., 2004). Within the nucleus it was originally identified as a non-histone DNA binding protein, which is loosely associated with chromatin and is involved in maintaining nucleosome structure, regulating gene transcription, and modulating the transcriptional activity of steroid hormone receptors including the glucocorticoid receptor (Gerlitz et al., 2009).

2.2. Passive release as a DAMP

HMGB1 is released from cells through two primary mechanisms, one involving passive release from necrotic or damaged cells and the other involving active secretion from immuno-competent cells. A seminal paper by Scaffidi and colleagues definitively showed that HMGB1 functions as a DAMP when it is passively released by cells undergoing necrotic cell death (Scaffidi et al., 2002). Under necrotic conditions, contents of the cytosol are dispersed into the extra-cellular space due to cellular distress or damage. Scaffidi found that in necrotic cells, HMGB1 dissociated from chromatin and was released from cells to induce a pro-inflammatory response. However, HMGB1 remained bound to chromatin and failed to induce a pro-inflammatory response in cells undergoing apoptotic or programmed cell death, in which cytosolic contents are sequestered and not “seen” by innate immune cells. In light of the finding that HMGB1 is released upon necrotic, but not apoptotic cell death as well as similar findings, Kono and Rock have proposed the “hidden self” model of immunogenicity. This model proposes that DAMPs are only “seen” under conditions of necrosis or cellular damage, wherein DAMPs are no longer sequestered within the cell and can alert the innate immune system to pathological cell conditions (Kono and Rock, 2008).

2.3. Active Secretion

The passive release described above can occur from virtually any cell type. However, HMGB1 is also released by innate immune cells under conditions that do not involve necrotic cell death. In response to pro-inflammatory stimuli such as LPS, HMGB1 is transported out of the nucleus and acts as an extracellular signaling molecule to modulate and induce a variety of inflammatory processes. This active HMGB1 secretion occurs through a two-step process. First, HMGB1 is translocated out of the nucleus to the cytoplasm after JAK/STAT1-regulated hyper-acetylation of lysine residues located in the A and B box domains (Lu et al., 2014). Once in the cytoplasm, HMGB1 is actively secreted by immuno-competent cells such as monocytes and macrophages. Of note, we have found that

microglia also actively secrete HMGB1 in response to acute stress (see section 3.4. below). HMGB1 is not released via the canonical endoplasmic reticulum/Golgi pathway, but the mechanism by which active secretion occurs remains unresolved. HMGB1 has been reported to accumulate into secretory lysosomes suggesting a role for Ca^{2+} regulated secretion (Gardella et al., 2002). Furthermore, formation of the NLRP3 inflammasome and activation of caspase-1 have also been implicated as regulatory mechanisms of HMGB1 release (Lamkanfi et al., 2010) and so HMGB1 may be released in a manner similar to interleukin-1β. Once released into the extra-cellular space, HMGB1 is thought to signal through the pattern recognition receptors (PRRs), TLR2 and TLR4, and the receptor for advanced glycation end products (RAGE)(Yang and Tracey, 2005). Activation of RAGE initiates two primary pathways leading to cell migration and proliferation as well as pro-inflammatory cytokine synthesis. One of these pathways leads to activation of the guanosine triphosphates CDC42 and RAC, which regulate cell motility and neurite outgrowth, while a second pathway involves phosphorylation of several mitogen-activated protein kinases (MAPKs) that activate the downstream transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Activation of TLR2 and TLR4 recruits MyD88 to activate similar MAPKs and subsequently, NF-κB (Yang et al., 2005). Therefore, each receptor target plays a role in HMGB1 signaling, although the relative contributions of each receptor to a cells inflammatory output are still under investigation and likely depend on the inflammatory event, cell-type and local microenvironment. It is important to note here that HMGB1 as an endogenous DAMP utilizes PRRs, TLR2 and TLR4, which are respectively the cognate receptors for the exogenous DAMPs (PAMPs) lipoteichoic acid (gram-positive bacterial motif) and LPS (gram-negative bacterial motif). Matzinger raises the provocative notion that PRRs such as TLRs may have originally evolved as receptors for endogenous DAMPs and that subsequently microbes co-opted these same PRRs to enhance their survival (Matzinger, 2002). This notion may help explain the molecular “promiscuity” of TLRs.

2.4. Redox state and function

There is an additional level of complexity with regard to HMGB1 signaling beyond the multiplicity of receptors it can ligate. Recent studies have found that the redox state of HMGB1 is a key determinant of its receptor interactions and immunological function. HMGB1 contains three critical cysteine residues (C23, C45, and C106) that are the site of post-translational modification (oxidation) to create three distinct redox forms of HMGB1, each with unique functional properties (Antoine et al., 2014; Venereau et al., 2012). While these redox forms are not detectable using antibody-based methodologies, the analytical technique of liquid chromatography-mass spectrometry has enabled researchers to determine the redox modification of specific amino acids, which have been related to specific biological functions (Yang et al., 2013). The predominant redox form of HMGB1 is a fully reduced form with each cysteine in a sulfhydryl state, also known as fully-reduced HMGB1 (frHMGB1)(See Fig. 1 inset). This form is present under basal conditions and is primarily located in the nucleus and cytoplasm. Under inflammatory conditions, frHMGB1 is released and forms a heterocomplex with the chemokine CXCL12 to interact with the chemokine receptor CXCR4 to promote cell migration, but not pro-inflammatory cytokine transcription or secretion (Schiraldi et al., 2012). The second form of HMGB1 is pro-inflammatory and occurs when a disulfide bond is formed between C23 and C45 under oxidizing conditions,

while C106 remains in a reduced state, also known as disulfide HMGB1 (dsHMGB1). There is some evidence that HMGB1 is oxidized in the cytoplasm as a result of ROS production (Kazama et al., 2008), which provides an intra-cellular microenvironment to modulate the redox state of HMGB1 and render HMGB1 pro-inflammatory. This molecular configuration of HMGB1 signals through TLR4 to induce synthesis and secretion of pro-inflammatory cytokines. A recent study shows that dsHMGB1 must bind to MD-2, an extra-cellular adaptor molecule of the TLR4 signalosome, to induce TLR4 signaling and subsequent pro-inflammatory cytokine synthesis (Yang et al., 2015). Other redox forms (frHMGB1 and oxHMGB1) exhibited negligible binding to MD-2 and were incapable of inducing TLR4 signaling. Of note, dsHMGB1 is sufficient to prime the microglial pro-inflammatory response to an immune challenge (see section 3.4. below), which is consistent with the priming qualities of HMGB1 in peripheral macrophages (Valdes-Ferrer et al., 2013). The last form of HMGB1 occurs under a state of complete oxidation wherein each cysteine is fully oxidized to a sulfonyl form and is not associated with any biological function (oxHMGB1). Interestingly, the half-life of frHMGB-1 is quite short (~17 mins in serum) compared with the half-life of dsHMGB1 (~8 hrs)(Zandarashvili et al., 2013). This redox sensitivity renders HMGB1 a highly labile immunologic molecule, which permits HMGB1 to rapidly transition between functional states depending upon the oxidizing nature of a microenvironment or biological compartment. This labile quality of HMGB1 also extends to its capacity to function as an endogenous adjuvant.

2.5. Endogenous adjuvant

HMGB1 exhibits adjuvant-like properties by forming complexes with a variety of exogenous and endogenous inflammatory stimuli and amplifies the pro-inflammatory impact of these stimuli (Rock et al., 2005). This conclusion stems from the observation that administration of recombinant HMGB1 does not reliably induce inflammation, yet HMGB1 neutralizing antibodies or antagonists reduce the inflammatory response to a variety of pro-inflammatory stimuli. Indeed, HMGB1 complexes with PAMPs including LPS and Pam3CSK4 (gram-positive bacterial motif), ssDNA as well as pro-inflammatory cytokines and chemokines including IL-1β and CXCL12. The complexes bind to the receptor targets for the non-HMGB1 partner and induce an exaggerated immune response compared with that to the ligand alone (Bianchi, 2009). For example, HMGB1 is capable of forming a complex with IL-1β. This complex then signals through the IL-1R1 receptor resulting in an amplification of IL-1β effects. Indeed, as Bianchi notes “HMGB1 loves company”, an attribute that may play a pivotal role in neuroinflammatory priming.

3. HMGB1 and neuroinflammation

In the brain, HMGB1 has been localized to microglia and astrocytes and is ubiquitously expressed at high levels in neurons. It has been implicated in the pathophysiology of several neurodegenerative diseases and its role in these processes has been reviewed previously (Fang et al., 2012). Here, our focus will be on a set of representative studies demonstrating a casual role for HMGB1 across a range of non-degenerative neuroinflammatory conditions, which illustrate the prominent role of neuronal HMGB1 as an initiator and amplifier of neuroinflammation as well as its role in neuronal excitation.

3.1. Cerebral ischemia and traumatic brain injury (TBI)

A number of studies have consistently shown that induction of an ischemic event in the brain leads to the release of HMGB1 (Yang et al., 2010), presumably from damaged neurons, which then functions as a DAMP. Interestingly, HMGB1 was found to rapidly translocate from the nucleus to the cytoplasm and then was released into the extracellular space. Notably, total HMGB1 protein levels in the ischemic hemisphere decreased suggesting that HMGB1 was either degraded or released into the periphery. Indeed, Kim and colleagues demonstrated that HMGB1 protein levels decreased in ischemic tissue while HMGB1 increased in CSF as well as serum suggesting that HMGB1 was released from ischemic brain tissue into the periphery (Kim et al., 2006). Moreover, using shRNA-mediated silencing of HMGB1 gene expression, Kim and colleagues demonstrated that HMGB1 mediated ischemia-induced infarct size, microglia activation, microgliosis, and pro-inflammatory cytokine increases. Consistent with these findings, Liu and colleagues also found that blocking HMGB1 signaling, in this case using a neutralizing antibody, reduced ischemia-induced neuroinflammatory processes, microglia activation, infarct size and neurological deficits (Liu et al., 2007). A recent study by Liesz and colleagues showed that cerebral ischemia induced the release of HMGB1 from ischemic tissue into serum and that HMGB1 was in a disulfide redox state (dsHMGB1), which is the pro-inflammatory form of HMGB1 (Liesz et al., 2015). Of note, HMGB1 became oxidized after release from brain and induced peripheral cytokines as well as sickness behavior via the RAGE receptor.

As with an ischemic event in the CNS, traumatic brain injury (TBI) results in direct damage to cerebral tissue and the release of alarmins and induction of neuroinflammatory responses (Corps et al., 2015). For example, TBI has been found to induce the translocation of HMGB1 from nucleus to cytoplasm, specifically in neurons (Okuma et al., 2012). As with the ischemia studies discussed above, there was a depletion of HMGB1 from the site of injury, presumably due to the release of HMGB1 into the extra-cellular space where it could function as a DAMP. Okuma and colleagues found that treatment with a neutralizing antibody to HMGB1 given intravenously reduced TBI-induced neuronal cell death, lesion volume, pro-inflammatory mediators, and neurological deficits, suggesting that HMGB1 was indeed released by damaged or distressed neurons. Similarly, Laird and colleagues showed that TBI resulted in the release of HMGB1 from necrotic neurons and that TLR4 mediated TBI-induced neuroinflammation (Laird et al., 2014), presumably due to the release of HMGB1 from necrotic neurons.

3.2. Seizure

Neuroinflammatory processes have also been implicated in the pathophysiology of seizure, which is associated with CNS injury (Vezzani, 2014). The damaging effects of seizure have thus focused research efforts on the role of HMGB1. Maroso and colleagues found that HMGB1 was increased in epileptogenic human brain tissue and in chronic epileptic mice (Maroso et al., 2010). Further, using the box A fragment of HMGB1 to block HMGB1 signaling, Maroso and colleagues found that HMGB1 mediated kainate-induced seizures through TLR4, which was co-localized with neuronal and astrocyte antigens, but not microglia antigens. In addition, recombinant HMGB1 administered into the hippocampus potentiated the proconvulsant effects of subsequent kainate treatment. These effects of

recombinant HMGB1 were abrogated in mice lacking functional TLR4 receptors. Of note, the redox state of HMGB1 was not characterized in this study. To further characterize the mechanism of HMGB1 induced seizure, Maroso and colleagues found that exposure of neuron and glia primary co-cultures to high concentrations of glutamate induced HMGB1 to translocate from the nucleus to cytoplasm of neurons. HMGB1 was subsequently released into the medium after prolonged glutamate exposure resulting in excitotoxic cell death. Consistent with these findings, Balosso and colleagues recently found that the disulfide form of HMGB1 increased NMDA-induced neuronal cell death and potentiated kainate-induced seizures (Balosso et al., 2014).

Interestingly, seizure induces the production and release of the pro-inflammatory cytokine IL-1β as well as activation of the NLRP3 inflammasome (i.e. caspase-1 activity) and IL-1β is a pivotal mediator of seizure activity (Maroso et al., 2011). Thus, there is the distinct possibility that HMGB1 may amplify the proconvulsant effects of IL-1β by complexing with IL-1β and enhancing signaling through the IL-1R1 receptor.

3.3. Ethanol exposure

A number of studies have shown that chronic ethanol exposure is also neuroinflammatory (Szabo and Lippai, 2014). Interestingly, chronic ethanol treatment has also been found to induce the neuronal release of HMGB1, which mediated the pro-inflammatory effects of ethanol (Zou and Crews, 2014). Interestingly, ethanol induced the acetylation of HMGB1 and subsequent extra-cellular release of HMGB1, which exerted its pro-inflammatory effects through TLR4. Zhou and Crews also found that ethanol induces NLRP3 expression in neurons and astrocytes and that NLRP3 expression is increased in neurons and astrocytes of post-mortem alcoholic brains (Zou and Crews, 2012). Further, ethanol induces IL-1β, which mediated ethanol inhibition of neurogenesis suggesting that ethanol activates the NLRP3 inflammasome. Zhou and Crews propose that ethanol induces the active release of HMGB1 from neurons, which signals through TLR4 to induce pro-inflammatory cytokines (Zou and Crews, 2014). These findings raise the possibility that other drugs of abuse (e.g. methamphetamine, morphine), which are also neuroinflammatory, may induce cellular distress or damage, thereby releasing HMGB1 and initiating/amplifying a neuroinflammatory cascade.

3.4. Stress-induced neuroinflammatory priming

Taken together, the data reviewed above indicate that HMGB1 plays a pivotal role in an array of neuroinflammatory conditions. A considerable number of studies have also shown that stress primes as well as induces neuroinflammatory processes (Frank et al., 2013). Briefly, a primed neuroinflammatory response occurs when exposure to a prior stimulus, in this case acute or chronic stress, potentiates the neuroinflammatory response to a subsequent pro-inflammatory stimulus such as LPS. For example, we have found exposure to inescapable tailshock potentiates the neuroinflammatory and sickness response (e.g. fever) to a subsequent immune challenge (LPS) given 24h after stress exposure (Johnson et al., 2002; Johnson et al., 2003; Johnson et al., 2004). Moreover, exposure to this stressor also potentiated the microglia pro-inflammatory response to LPS ex vivo suggesting that microglia are a CNS substrate for stress-induced priming (Frank et al., 2007; Frank et al.,

2012; Weber et al., 2013; Weber et al., 2015). Similar neuroinflammatory priming effects have been observed with chronic stressors. Wohleb and colleagues found that six consecutive days of chronic social defeat stress potentiated the microglial pro-inflammatory response to LPS ex vivo (Wohleb et al., 2011). Likewise, chronic unpredictable stress involving a regimen wherein animals are exposed to a different stressor (e.g. forced swim, restraint, water deprivation, food deprivation, cold stress, circadian disruption) each day over a 1 – 2 week time period also primes the neuroinflammatory response to a subsequent immune challenge (de Pablos et al., 2014; de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Munhoz et al., 2006). Indeed, several stress-induced mediators have been implicated in neuroinflammatory priming including glucocorticoids (Frank et al., 2012), catecholamines (Wohleb et al., 2011) and pro-inflammatory cytokines (Johnson et al., 2004). Interestingly, TLR2 and TLR4 have been implicated in stress-induced neuroinflammatory processes as well (Weber et al., 2013). For these reasons, we have recently explored whether HMGB1 plays a significant role in these processes given that HMGB1 is an endogenous ligand for TLR2 and TLR4.

Initially, we found that exposure to a series of tailshocks increased HMGB1 protein levels in the hippocampus immediately after the stress session as well as 24hr later (Weber et al., 2015). Further, we found that hippocampal microglia, isolated immediately after stress exposure, exhibited increased secretion of HMGB1 compared to control animals. Importantly, cell viability did not differ between stress and control groups suggesting that HMGB1 was actively secreted by microglia and not passively released due to cell necrosis. It should be noted that these data do not exclude the possibility that stress induced the release of HMGB1 from other cell types (e.g. neurons, astrocytes) either via passive (necrosis) release or active secretion. Clearly, the studies on ischemia, TBI, seizure and ethanol discussed above indicate that neurons are a significant source of HMGB1. Therefore, a likely possibility is that neurons are also significant source of HMGB1 in stress.

In light of the effects of stress on HMGB1, it was of interest to determine whether HMGB1 played a role in stress-induced priming of the microglia pro-inflammatory response. Towards this aim, the box A fragment of HMGB1, which functions as a competitive antagonist of HMGB1, was injected intra-cisterna magna (ICM) immediately prior to stress exposure to block stress-induced priming of microglia. As with prior studies (Frank et al., 2013), microglia were isolated from hippocampus 24h after stress exposure and were challenged with LPS to probe for stress-induced priming. Indeed, box A blocked the effect of stress on microglia priming, suggesting that HMGB1 mediates the priming effects of stress on microglia. Further, this effect of box A provided additional evidence that stress leads to the release of HMGB1 within the CNS. However, it was unclear what redox form of HMGB1 (i.e. dsHMGB1 and/or frHMGB1) mediated stress-induced priming because box A presumably blocks both molecular forms and HMGB1 antibodies do not distinguish between these redox forms (Weber et al., 2015).

To begin to address this question, dsHMGB1 or frHMGB1 was injected ICM and microglia priming was assessed (Weber et al., 2015). In this experiment, microglia were isolated from hippocampus 24 hr after injection of these redox forms of HMGB1. Microglia were then treated with LPS to probe for priming of the pro-inflammatory response. We found that

dsHMGB1 primed the pro-inflammatory response of microglia, whereas frHMGB1 failed to prime microglia, suggesting that dsHMGB1 is most likely the redox form of HMGB1 released during stress and that primes microglia. These effects of dsHMGB1 and frHMGB1 are consistent with observations that dsHMGB1 is pro-inflammatory, whereas frHMGB1 is chemotactic and not pro-inflammatory (Venereau et al., 2012).

Two key questions arise from these findings. One pertains to the mechanism of HMGB1 secretion that is engaged by stress exposure, and the other to the mechanism by which HMGB1 primes microglia. As to the latter question, we found that stress as well as dsHMGB1 increased expression of NLRP3 (Weber et al., 2015) suggesting that HMGB1 induction of NLRP3 may play a pivotal role in the effects of stress on microglia priming. These findings are consistent with a recent study that also found that stress increases expression of NLRP3 (Pan et al., 2014). Briefly, NLRP3 is an inflammasome component that mediates, in large part, processing and maturation of IL-1β (Martinon et al., 2009). Inflammasomes are multi-protein complexes that are involved in the activation of inflammatory caspases, which play a pivotal role in the processing and maturation of pro-inflammatory cytokines. Importantly, the NLRP3 inflammasome is a sensor for a diverse array of endogenous and exogenous danger signals and has been implicated in the pathophysiology of sterile inflammatory diseases (Leemans et al., 2011). Formation and activation of the NLRP3 inflammasome requires both a priming step and a second activation step (Hornung and Latz, 2010), which is a property unique to the NLRP3 inflammasome. Priming of the NLRP3 inflammasome is induced by a stimulus that signals, in part, through TLR4 to increase NLRP3 expression to a critical level necessary for inflammasome formation. If the inflammasome is then exposed to a subsequent activating signal, the primed increase in NLRP3 permits formation of a molecular complex with the adaptor ASC, which recruits and cleaves pro-caspase-1 to mature caspase-1. Finally, caspase-1 converts pro-IL-1β to the mature and active form of IL-1β, which is released into the extra-cellular space.

The effects of stress on HMGB1 and NLRP3 as well as the mediating role of HMGB1 in microglia priming, suggest a mechanism whereby stressor exposure can induce priming and is depicted in Fig. 1. We propose that stress induces an increase as well as active secretion of HMGB1 from microglia, although other cell types, in particular neurons, are not excluded. Extra-cellular HMGB1, which we propose is the dsHMGB1 form, then primes microglia by paracrine/autocrine signaling through TLR4 to increase NLRP3 expression to a critical level that permits formation and activation of the NLRP3 inflammasome *if a subsequent immune challenge (e.g. LPS) occurs*. It should be noted that other cognate receptors for HMGB1 including TLR2 and RAGE may be involved in microglia priming.

Interestingly, stress hormones such as glucocorticoids (GCs) have been implicated in NLRP3 inflammasome priming (Busillo et al., 2011). Busillo and colleagues found that GCs increase NLRP3 mRNA and protein in macrophages without inducing a pro-inflammatory response, an effect mediated by the GC receptor. If GC-exposed macrophages were subsequently challenged with an NLRP3 inflammasome-activating stimulus (ATP), the pro-inflammatory response to ATP was amplified suggesting that GCs primed macrophages by increasing expression of NLRP3. We have also found that GC treatment of isolated

microglia increases NLRP3 expression (unpublished observations). However, it is unclear how GCs induce NLRP3. One possibility is that GCs directly induce NLRP3 transcription via GC receptor transactivation in the promoter region of NLRP3, but this possibility awaits experimental testing. Alternatively, we propose that GCs induce NLRP3 indirectly through the release of HMGB1.

A number of studies suggest that GCs play a pivotal role in stress-induced potentiation of the neuroinflammatory response to a subsequent immune challenge (de Pablos et al., 2014; de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Munhoz et al., 2006). These studies repeatedly administered a GC receptor antagonist (RU486) during chronic stress exposure and found that blockade of stress-induced GC signaling blocked the neuroinflammatory priming effects of stress. Similarly, we found that GCs mediate the neuroinflammatory priming effects of an acute stressor (Frank et al., 2012) Here, RU486 was administered ICM immediately prior to stress (~2h of inescapable tailshock) exposure. 24h after termination of the stressor, animals were either given a peripheral immune challenge with LPS or hippocampal microglia were isolated and directly challenged with LPS. Consistent with the chronic stress studies cited above, blockade of stress-induced GC signaling ameliorated stress-induced priming of the neuroinflammatory and microglia pro-inflammatory response to LPS. Similar effects were observed in adrenalectomized animals. To determine whether GCs are sufficient to prime microglia *in vivo*, we found that a single injection of GCs administered 24 hr prior to immune challenge (i.e. LPS) potentiated the neuroinflammatory and microglia pro-inflammatory response to LPS (Frank et al., 2010). Of note, the dose of GC used resulted in serum GC levels that mimic stress-induced GC levels. Moreover, we found that if the same dose of GC was administered 1 hr *after* an immune challenge with LPS, the neuroinflammatory response was suppressed by GCs. These results suggested that the timing of stress and GC exposure relative to an immune challenge is a critical factor underpinning the priming effects of stress and GCs. Moreover, the interval between GC exposure and a subsequent immune challenge is also a critical factor involved in neuroinflammatory priming such that a 24 hr, but not a 2 hr interval was sufficient to prime the neuroinflammatory response. Similar results were observed with chronic GC exposure (Frank et al., 2014). Animals were treated with varying concentrations of GCs in drinking water for 10 days. The high GC treatment condition potentiated the microglia pro-inflammatory response to LPS. Further, high GC treatment increased expression of NLRP3 in hippocampus and potentiated the LPS induction of NLRP3 in microglia. In light of these findings and that GCs prime the NLRP3 inflammasome (Busillo et al., 2011), we propose that stress-induced increases in central GCs results in activation of GC receptors and, through an unknown mechanism, subsequent increase and secretion of HMGB1 within the CNS, which then primes the NLRP3 inflammasome.

We speculate that if GCs reach a critical threshold as during a fight/flight response, they may thus function as an alarmin by inducing HMGB1, thereby preparing an organism's innate immune system (NLRP3 inflammasome priming) for subsequent immune challenges such as injury, trauma or infection, which are more likely to occur during a fight/flight response. In doing so, GCs may confer a significant survival advantage by enhancing the central innate immune and sickness response to immune challenges.

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- HMGB1 is a danger-associated molecular pattern with unique structure and function
- HMGB1 plays a pivotal role in the pathophysiology of neuroinflammatory conditions
- HMGB1 mediates stress-induced neuroinflammatory priming
- HMGB1 mediated priming may involve the NLRP3 inflammasome
- Glucocorticoids may play a pivotal role in stress- and HMGB1-mediated priming

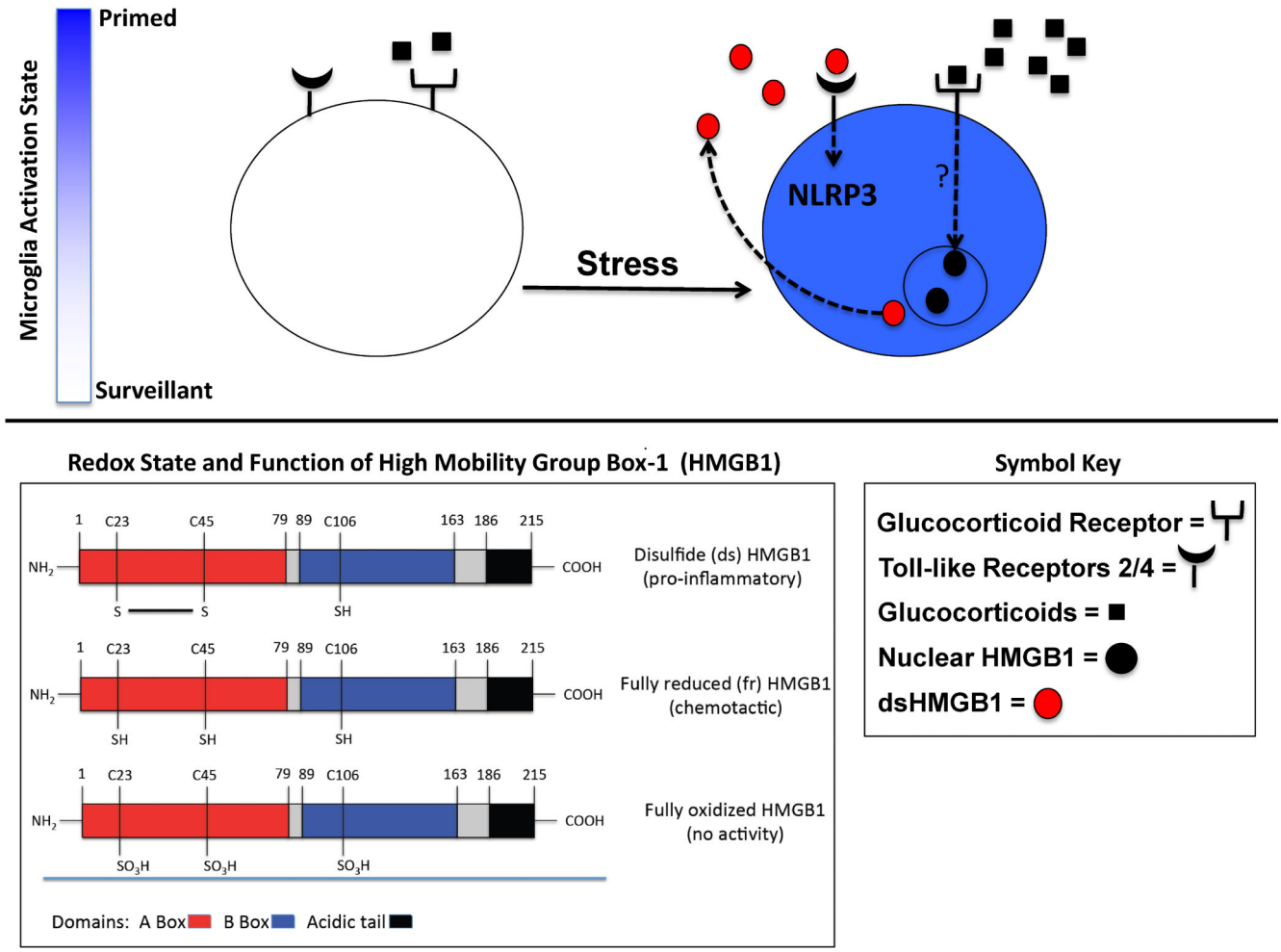

Fig. 1.

A model of stress- and HMGB1-mediated neuroinflammatory and microglia priming. We propose that the following steps occur in the process of stress-induced microglia priming. First, exposure to a stressor induces central increases in glucocorticoids (GCs). If GCs reach a critical threshold in brain, the GC receptor (GR) is engaged resulting in the synthesis and active release of HMGB1. The question mark denotes that the mechanism of GC/GR action on HMGB1 is unknown. HMGB1 is then actively secreted from microglia and possibly other cell types in the disulfide form (dsHMGB1). dsHMGB1 then engages TLR2/TLR4 on microglia resulting in priming of the NLRP3 inflammasome and thus shifting the microglia phenotype towards a primed state. Finally, if a subsequent immune challenge occurs, the neuroinflammatory and microglia pro-inflammatory response will be potentiated.