Danger Signals and Inflammasomes: Stress-Evoked Sterile Inflammation in Mood Disorders

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Major depressive disorder (MDD) and other mood disorders remain difficult to effectively treat, and innovative interventions and therapeutic targets are needed. Psychological stressors and inappropriate inflammation increase the risk and severity of mood disorders; however, only recently have the importance of sterile inflammatory processes in this effect been revealed. This review will introduce the reader to pathogen vs sterile inflammation, inflammatory receptor–ligand interactions, microbial-associated molecular patterns (MAMPs), pathogen-associated molecular patterns (PAMPs), danger-associated molecular patterns (DAMPs), and the more recent discovery of the role of the inflammasome in peripheral and central nervous system cytokine/chemokine inflammatory responses. The review will focus on current preclinical and clinical evidence that sterile inflammation and inflammasome-dependent signaling may contribute to mood disorders. By understanding these inflammatory signaling processes, new approaches for quieting chronic or inappropriate inflammatory states may be revealed and this could serve as novel pharmacological targets for the treatment of mood disorders.

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INTRODUCTION

Inflammatory states in the body and/or central nervous system (CNS) impact a variety of brain functions including mood (Miller and Raison, 2015). There is strong evidence that patients with major depressive disorder (MDD), for example, have elevated levels of inflammatory proteins in the blood, and many inflammatory diseases are associated with increased rates of MDD (Howren *et al*, 2009; Schiepers *et al*, 2005). In addition, repeated or severe stressor exposure, in the absence of pathogenic disease, increases affective dysregulation and inflammatory proteins in tissues and blood in humans (Bierhaus *et al*, 2003; Pace *et al*, 2006) and animals (Maslanik *et al*, 2012a, b). The stress-evoked cytokine/chemokine response is an example of sterile inflammation (Fleshner, 2013). Although immunologists have long recognized the adaptive nature of this local tissue response after injury, it has only recently been appreciated that a psychological or systemic stressor in the absence of overt tissue damage can also trigger systemic and CNS sterile inflammation and that in some contexts this can be detrimental to physical and mental health.

This review will introduce the reader to pathogen vs sterile inflammation, inflammatory receptor–ligand interactions, microbial-associated molecular patterns (MAMPs), pathogen-associated molecular patterns (PAMPs), danger-associated molecular patterns (DAMPs), and the more recent discovery of the role of the inflammasome in body and CNS cytokine/chemokine inflammatory responses. The review will focus on current preclinical and clinical evidence that sterile inflammation and inflammasome-dependent signaling may contribute to mood changes. By understanding these inflammatory signaling processes, new approaches for quieting chronic inflammatory states may be revealed, and could serve as novel pharmacological targets for treating mood disorders.

Clinical Evidence Linking Inflammation and Stress-Related Mood Disorders

Compelling clinical research has established that repeated, chronic and excessive stressor exposure increases inflammatory state and is associated with increased risk and severity of a variety of mood disorders (for reviews see Kiecolt-Glaser *et al*, 2010, 2015; Segerstrom and Miller, 2004; Slavich and Irwin, 2014). For example, exposure to repeated life stressors or laboratory stressors involving social conflict, threat, isolation, and rejection increases inflammatory markers including C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1β

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Figure 1. Substantial overlap between pathogen-associated molecular pattern (PAMP) and stress-evoked, likely danger-associated molecular pattern (DAMP), inflammatory protein changes. This schematic is based on results from a series of studies measuring inflammatory proteins in blood and peripheral tissues (spleen, liver) using multiplex enzyme-linked immunosorbent assays (ELISAs) and mRNA using quantitative real-time PCR (qRT-PCR) after lipopolysaccharide (LPS) (PAMP) or tailshock.

(IL-1 β) and soluble tumor necrosis factor- α receptor (TNFsr) in the blood (Aschbacher *et al.*, 2012; Denson *et al.*, 2009; Dickerson *et al.*, 2009; Steptoe *et al.*, 2007) and NF- κ B mRNA (a transcription factor for inflammatory proteins) in leukocytes (Murphy *et al.*, 2013; Pace *et al.*, 2012). This elevated inflammatory state has also been associated with increased risk and severity of mood disorders including depression (Aschbacher *et al.*, 2012; Danese *et al.*, 2008; Pace *et al.*, 2012).

An acute excessive stressor or a single major life event can also increase inflammatory markers. For example, Schultze-Flore *et al.* (2012) reported that the death of a spouse increased IL-1 β and IL-6 activity in older adults. Interestingly, increases in inflammatory markers following exposure to an acute traumatic event may be predictive of the development, symptom severity, and duration of mood disorders such as depression, anxiety, and post-traumatic stress disorder (PTSD; for a review see Felger *et al.*, 2016). Pervanidou *et al.* (2007), for example, reported that children with higher concentrations of plasma IL-6 following a motor vehicle accident had a greater chance of developing PTSD compared with children who did not have elevated IL-6 after the accident or controls. Furthermore, Michopoulos *et al.* (2015) and Heath *et al.* (2013) reported that CRP concentration was correlated with PTSD symptom severity and duration, such that PTSD patients with high, compared with low, CRP had greater symptom severity and duration (Heath *et al.*, 2013; Michopoulos *et al.*, 2015). There is, therefore, a growing body of clinical studies establishing the association between stressor exposure, inflammation, and mood disorders. This review will focus on exciting advances in our understanding of the nature of signals and cellular sources responsible for stress-evoked increases in inflammatory state and will reveal novel future therapeutic targets to reduce stress-evoked elevation of inflammation and mood disorders.

Pathogen vs Sterile Inflammation

Innate immune cells are the primary effectors of the inflammatory response, although adaptive immune cells

such as Th1 cells are important collaborators. These innate immune cells are found throughout the body and CNS and respond to pathogenic challenges, cellular stress, tissue damage, and tissue repair. Receptor–ligand recognition schema is an important primary distinguishing characteristic between adaptive and innate immune cells. Cells of the innate immune system use germline-encoded receptors designed to recognize conserved molecular patterns and have been coined pattern recognition receptors (PRRs). The nature of the molecular patterns capable of binding PRRs is vast and include those commonly associated with microbes in general (MAMPs). MAMPs encompass both pathogenic and commensal/symbiotic microbes. Those patterns associated with microbes that are typically pathogenic to the host have been coined PAMPs. Examples of MAMPs and PAMPs include lipopolysaccharide (LPS, a membrane-integrated component of many Gram-negative pathogenic bacteria) or CpG DNA (a common viral motif). Importantly, a robust inflammatory response is the consequence of PRR-PAMP binding.

Ligands associated with cellular stress and tissue damage have been coined DAMPs. In contrast to PAMPs and MAMPs, which are associated with microbes and viruses, DAMPs are endogenous molecules derived from self and are increased after cellular stress and tissue damage. In most instances these self-molecules only function as DAMPs when they are in the extracellular environment because of stress-evoked release and/or necrotic cell death. The consequence of PRR-DAMP binding is sterile inflammation (Kono and Rock, 2008; Rock *et al.*, 2010). Newly identified potential DAMPs are rapidly emerging in the literature, including extracellular heat-shock protein 72 (Hsp72), uric acid crystals, high-mobility group box 1 (HMGB1), and adenosine triphosphate (ATP), discussed below.

There is considerable overlap in a broad number of inflammatory proteins that are modulated after either LPS (PAMP) or stressor exposure (DAMP) and that are detectable in the blood and peripheral tissues (ie, adipose, liver, spleen, lymph nodes). Figure 1 depicts the overlap of cytokines, chemokines, and anti-microbials (mRNA or protein) after either LPS (Campisi *et al.*, 2003b; He *et al.*, 2014) or a well-established acute stressor (100, 5-s, inescapable tailshocks (Maslanik *et al.*, 2012a, b)). Brain tissue and microglia also respond to PAMPs and DAMPs and increase inflammatory proteins as described below.

Stress, DAMPs, and Sterile Inflammation

Local sterile inflammation after tissue injury and necrotic cell death has an important role in tissue repair (Rock *et al.*, 2011), whereas systemic sterile inflammation or the cytokine storm triggered after major trauma can be lethal (Schneider *et al.*, 2011). Recently, it has been reported that psychological and/or acute intense stressor exposure in the absence of overt tissue damage can evoke a detectable local and systemic sterile inflammatory response and that DAMPs may have a

role (Fleshner, 2013; Frank *et al.*, 2015b; Maslanik *et al.*, 2013; Miller *et al.*, 2015).

Fleshner and co-workers reported in a series of studies that rats exposed to uncontrollable tailshock (Campisi and Fleshner, 2003a; Campisi *et al.*, 2012; Maslanik *et al.*, 2013), a predatory cat with no physical contact (Fleshner *et al.*, 2004), or predatory ferret odor (Figure 2), had increased blood levels of several DAMPs (ie, Hsp72, uric acid crystals) and little evidence of cell death (ie, low levels of LDH). It appears therefore that stressor exposure may stimulate DAMP release that is not dependent on necrotic cell death. In support of this idea is the observation that the release of Hsp72 (a possible DAMP) after tailshock is dependent on α -adrenergic, but not β - or glucocorticoid, receptor signaling (Johnson *et al.*, 2005; Johnson and Fleshner, 2006). Tailshock also increases tissue and blood concentrations of many cytokines and chemokines, and both MAMP (derived from the gut bacteria) and DAMP signals are involved (Maslanik *et al.*, 2012b, 2013). Importantly, in this series of studies it was also discovered that some features of the sterile inflammatory response after stressor exposure are dependent on the inflammasome, especially nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain protein 3 (NLRP3) (Maslanik *et al.*, 2013).

Inflammasomes are intracellular multiprotein complexes that function as sensors of DAMPs or PAMPs, which leads to the activation of proinflammatory caspases, and the cleavage and release of proinflammatory cytokines (Walsh *et al.*, 2014). Several distinct inflammasomes have been characterized, which generally consist of a cytosolic sensor (eg, a nucleotide binding domain), an adaptor protein (eg, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC)), and an effector caspase, typically caspase-1. Our focus here will be on the NLRP3 inflammasome as it has received the most attention and has an important role in peripheral and CNS inflammatory states stimulated by MAMPs, PAMPs, and/or DAMPs.

Unlike the activation of other inflammasomes, NLRP3 inflammasome formation and activation requires a two-step process. Figure 3 depicts both single signal (inflammasome-independent) and double signal (inflammasome-dependent) sterile inflammation. For cytokines that are inflammasome independent, the process begins with NF- κ B activation after MAMPs or DAMPs binding to TLR-4, CD14, and other potential PRRs. This binding stimulates inflammatory protein gene transcription, translation, protein synthesis, and release. In contrast, inflammasome-dependent cytokine synthesis and release is a two-step process that is initiated after ligation of TLRs and other receptors capable of binding DAMPs and/or MAMPs leading to NLRP3 gene transcription, translation, and protein production (Duewell *et al.*, 2010). Once sufficient NLRP3 protein has been formed and the cell is 'primed', a second activation signal triggers NLRP3 to interact with the adaptor protein ASC, which then recruits procaspase-1 through its caspase recruitment domain. This assembly of proteins is considered the inflammasome, and once formed triggers the activation/cleavage of procaspase 1.

Figure 2. The previously unpublished results of a study testing the effect of a psychological stressor, predatory order, on danger-associated molecular pattern (DAMPs) in the blood. Adult, male Sprague-Dawley rats (8 per group) had towels placed in their home cages that were saturated either with neutral control odor (strawberry) or a predatory odor (ferret urine) 10 min per day for 7 days. Serum levels of heat-shock protein 72 (Hsp72) were measured using enzyme-linked immunosorbent assay (ELISA).

Mature, active caspase-1 then acts to cleave pro-IL-1 β into mature IL-1 β (and IL-18) protein. The NLRP3 inflammasome is also unusual in that it responds to a very broad range of signals as the second event that produces inflammasome formation/activation (eg, ATP, K⁺ efflux, β -amyloid, silica, uric acid crystals, ROS; Elliott and Sutterwala, 2015). In addition, there are several candidate receptors capable of binding DAMPs and MAMPs, including TLRs, RAGE, and CD91 (Asea *et al.*, 2002; Calderwood *et al.*, 2007a, b). This scenario has led to the suggestion that the NLRP3 inflammasome is 'a common response mechanism to perturbations of homeostasis in the broadest sense' (Bauernfeind *et al.*, 2012).

The NLRP3 inflammasome has been implicated in a diverse array of sterile inflammatory diseases including ischemia-reperfusion injury, autoinflammatory diseases, type 2 diabetes, gout and pseudogout, obesity, atherosclerosis, and Alzheimer's disease (Leemans *et al.*, 2011), and sterile inflammatory diseases such as these also have a high comorbidity with MDDs (Katon, 2011). In addition, there is evidence of activation of the NLRP3 inflammasome in patients with MDD (Alcocer-Gomez and Cordero, 2014a; Drago *et al.*, 2015). Specifically, patients with depression had increased expression of NLRP3 and caspase-1 in peripheral blood mononuclear cells (Alcocer-Gomez *et al.* (2014b)). Thus, sterile inflammation mediated by DAMPs and the inflammasome and evoked after exposure to psychological stressors may have a role in the pathogenesis of MDDs and other psychopathologies (Alcocer-Gomez and Cordero, 2014a; Kessler, 1997; Miller *et al.*, 2015).

Of note, many of these peripheral inflammatory processes have also been characterized in the CNS of animals exposed to stress or animal models of depression, which we address

Figure 3. Both single signal (inflammasome-independent) and double signal (inflammasome-dependent) sterile inflammation. Inflammasome-dependent cytokine synthesis and release is a two-step process that is initiated after ligation of Toll-like receptor (TLRs) and other receptors capable of binding danger-associated molecular patterns (DAMPs) and/or microbial-associated molecular patterns (MAMPs) leading to NLRP3 (nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain protein 3) gene transcription, translation, and protein production (Duewell *et al*, 2010). Once sufficient NLRP3 protein has been formed, then a second activation signal is needed that leads NLRP3 to interact with the adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), which then recruits procaspase-1 through its caspase recruitment domain. This assembly of proteins is considered the inflammasome, and once formed triggers the activation/cleavage of procaspase 1. Mature, active caspase-1 then acts to cleave pro-interleukin-1 β (IL-1 β) into mature IL-1 β (and IL-18) protein. The NLRP3 inflammasome responds to a broad range of signals, for example, ATP, K⁺ efflux, β -amyloid, silica, uric acid crystals, and reactive oxygen species (ROS) (Elliott and Sutterwala, 2015). In addition, there are several candidate receptors capable of binding DAMPs and MAMPs, including TLRs, RAGE, and CD91 (Asea *et al*, 2002; Calderwood *et al*, 2007a, b). This figure was adapted from a figure courtesy of Tom Maslaniuk, PhD/MBA.

in the following sections. It is important to note that peripheral inflammatory processes are capable of inducing neuroinflammatory processes through several well-characterized immune-to-brain signaling pathways (for a review see Capuron and Miller, 2011), which include humoral as well as neural pathways. For example, the humoral pathway may involve blood-borne cytokines inducing neuroinflammatory processes in the brain through active cytokine transport across the BBB, entry into the brain at circumventricular organs, in which the BBB is absent or weak, or binding of cognate receptors on brain endothelial cells, which leads to transduction of cytokine signaling into the brain. Alternatively, cytokines as well as PAMPs (eg, LPS) are capable of activating afferent vagal nerve fibers in the periphery, which leads to activation of neural pathways in brain regions implicated in motivation and mood (McCusker and Kelley, 2013). Once a peripheral inflammatory stimulus

signals the brain, microglia mediate, in large part, the neuroinflammatory response to that stimulus. Accordingly, the following sections will focus on this pivotal innate immune effector cell of the CNS.

Microglia and Neuroinflammation

Resident microglial cells largely mediate innate immunity in the CNS. Microglia are mononuclear phagocytes that occupy the brain parenchyma and are ontogenetically distinct from other CNS mononuclear phagocytes including meningeal, choroid plexus, and perivascular macrophages, which reside outside the brain parenchyma (Katsumoto *et al*, 2014). It is important to note that these macrophage subtypes also serve a critical role in the brain's innate immune response and may contribute to the processes under discussion here

(Schiltz and Sawchenko, 2003). Unlike other CNS macrophages, in the adult CNS microglia are maintained independent of circulating blood monocytes and are thought to self-renew from progenitor cells in the CNS (Katsumoto *et al.*, 2014). Microglia are normally in a quiescent state, during which their processes sample their immediate environment several times a second (Nimmerjahn *et al.*, 2005).

Microglia perform several critical functions in the CNS including immunosurveillance for pathogens, cellular debris, apoptotic cells, and alterations in neuronal phenotype (Ransohoff and Cardona, 2010). Recent reviews suggest that microglia may enter a spectrum of activation states (Mosser and Edwards, 2008; Rivest, 2009) characterized by varying blends of immunophenotypes and cytokine profiles. Importantly, microglia can enter a state called 'primed' (Perry *et al.*, 2007). Here, they show phenotypic signs of activation (eg, upregulated MHCII), but do not secrete increased cytokines. However, if stimulated while in this state, microglia produce and release exaggerated quantities of mediators, including IL-1 β . This primed activation state is particularly relevant to stress-induced priming of neuroinflammatory processes, which is discussed in detail below. It should be noted that microglia express PPRs such as TLRs, which include TLR2 and TLR4, and TLR ligation potently activates microglia (Aravalli *et al.*, 2007). In addition, several inflammasomes have been characterized in microglia including NLRP1, NLRP3, and NLRC4 (Walsh *et al.*, 2014). Notably, several studies have characterized inflammasomes in neurons (de Rivero Vaccari *et al.*, 2009; Kummer *et al.*, 2007), which are capable of producing IL-1 β (de Rivero Vaccari *et al.*, 2009). The NLRP3 inflammasome has been the most studied inflammasome in the CNS (Walsh *et al.*, 2014), and as expanded upon below, has also been the focus of the preponderance of studies into the role of inflammasomes in animal models of depression. Interestingly, recent investigations into the effects of stress on NLRP3 in the CNS suggest that NLRP3 may also be uniquely sensitive to the homeostatic perturbations produced by stress.

Animal Models of Depression: Role of Inflammasomes

A number of studies have demonstrated that IL-1 β mediates depressive-like behaviors induced by stress as well as proinflammatory challenges, which of course implicates a mechanistic role for inflammasomes because inflammasomes mediate, in large part, the processing and maturation of IL-1 β (Jo *et al.*, 2016). Further, when administered to naive animals, IL-1 β is sufficient to induce depressive-like behaviors (Goshen *et al.*, 2009). While these studies implicate inflammasome activation in stress-induced depressive-like behaviors, inflammasomes were, in large part, not directly studied. It is important to emphasize here that stress-induced changes in brain IL-1 β do not necessarily reflect inflammasome activation because inflammasomes are not strictly required for the processing of IL-1 β into its mature, active

form. Of note, several inflammasome-independent or non-canonical pathways have been characterized, which also result in caspase activation and maturation of IL-1 β (Netea *et al.*, 2015). Further, inflammasome-dependent activation of IL-1 β is cell type specific (Netea *et al.*, 2015). Interestingly, Burm *et al.* (2015) have recently demonstrated that IL-1 β activation is not strictly caspase-1 dependent in primary microglia, suggesting that inflammasomes may not be necessary for the processing of IL-1 β into its mature form in this CNS innate immune cell. These are important points to consider in studies that examine the role of brain inflammasomes in animal models of depression.

The role of inflammasomes in stress-induced depressive-like behaviors has recently become a subject of study. Pan *et al.* (2014) conducted one of the initial studies on the role of the NLRP3 inflammasome in stress-induced depressive-like behavior. Rats were exposed to 12 weeks of chronic unpredictable stress (CUS). Briefly, CUS typically involves the repetitive and unpredictable presentation of aversive stimuli/conditions, including cage tilting, water and food deprivation, wet bedding, light cycle disruption, strobe lighting, and continuous noise (Willner, 1997). Indeed, this paradigm is highly effective at inducing depressive-like behaviors such as reductions in sucrose preference and juvenile social exploration. Pan *et al.* (2014) found that CUS exposure suppressed sucrose intake relative to unstressed controls, while increasing the levels of IL-1 β mRNA and mature IL-1 β protein in the prefrontal cortex (PFC). Interestingly, CUS also increased NLRP3 protein and mRNA, and mature caspase-1 protein in the PFC. Moreover, these effects of CUS were significantly blunted by chronic fluoxetine treatment, which was administered during weeks 6–12 of CUS exposure. However, it is important to note that the correlative nature of these findings precludes definitive conclusions regarding a causal role for the NLRP3 inflammasome in CUS-induced depressive-like behaviors as well as neuroinflammatory processes. In addition, much the same changes have been reported in the hippocampus (Deng *et al.*, 2015a; Liu *et al.*, 2015) and PFC (Deng *et al.*, 2015a). Of note, CUS exposure also was found to increase protein levels of the NLRP3 adaptor protein ASC (Deng *et al.*, 2015a, b; Li *et al.*, 2015). As noted above, the current conceptualization of NLRP3 inflammasome activation suggests that both a priming stimulus and a subsequent activating stimulus are required to form and activate the NLRP3 inflammasome. However, it is not clear what mediators play the roles of the NLRP3 priming stimulus and activating stimulus during exposure to CUS. Thus, it is unclear how these effects of CUS on NLRP3, caspase-1, and mature IL-1 β fit into our current understanding of the function of NLRP3. Could it be that initial stress exposure primes NLRP3 inflammasome formation to subsequent stress exposure during CUS? One possibility is that the repetitive, unpredictable presentation of heterogeneous stressors during CUS may serve to both prime and activate the NLRP3 inflammasome.

A recent study by Cheng *et al.* (2016) may provide insight into this question. They found that exposure to an initial

series of acute footshocks increased NLRP3 as well as mature caspase-1 in the hippocampus. It would seem that IL-1 β should also have been increased given the stress-induced increase in mature caspase-1, but it was not. However, if a second series of footshocks was administered 24 h after the initial series of footshocks, IL-1 β protein was increased, suggesting that the initial series of shocks had primed the NLRP3 inflammasome. These findings further suggest that a similar priming phenomenon may occur during exposure to CUS. We have also found that exposure to acute stress, in this case inescapable tailshock, increases NLRP3 protein in the hippocampus up to 24 h after stress treatment; however, microglial IL-1 β was not increased at that time-point post stress (Weber *et al.*, 2015). Importantly, treatment of hippocampal microglia with the TLR4 agonist LPS resulted in a potentiated IL-1 β response in animals exposed to stress 24 h earlier, suggesting that the NLRP3 inflammasome was primed by initial stress exposure. Although these studies only show a correlation between the NLRP3 inflammasome and stress-induced neuroinflammatory processes and depressive-like behaviors, several studies have provided evidence for a causal role for the inflammasome in these processes.

Alcocer-Gomez *et al.* (2015) used NLRP3 knockout (KO) mice to study the role of NLRP3 in mediating the effects of chronic immobilization stress on neuroinflammatory processes and depressive-like behavior. Mice were exposed to a 2-h immobilization stress every day for 30 consecutive days. They found that exposure of wild-type mice to immobilization induced a decrease in sucrose preference and social interaction, and an increase in immobility in the forced swim test. This stress-induced depressive phenotype was completely abrogated in NLRP3 KO mice. Furthermore, KO of the *NLRP3* gene prevented the stressor treatment from increasing protein levels of mature IL-1 β in the PFC and hippocampus. In addition, Wong *et al.* (2016) recently reported that caspase-1-deficient mice (casp1 $^{-/-}$) were protected from developing anxiety- and depression-like behaviors after chronic restraint stress (Wong *et al.*, 2016). These findings suggest that the NLRP3 inflammasome may have a causal role in stress effects on depressive-like behavior.

A recent investigation by Iwata *et al.* (2015) also found that 21 days of CUS resulted in a depressive behavioral phenotype including decreased sucrose preference, which was abrogated in NLRP3 KO mice. In wild-type mice, they also found that peripheral administration of an IL-1 β -blocking antibody ameliorated the behavioral effects of CUS, including decrements in sucrose preference and immobility in the forced swim test. These findings suggest that stress-induced activation of the NLRP3 inflammasome and subsequent maturation and release of IL-1 β was necessary for the depressive-like behavioral effects of stress. Iwata *et al.* (2015) also explored the role of ATP, a well-characterized activator of the NLRP3 inflammasome (Elliott and Sutterwala, 2015), in stress-induced activation of NLRP3, IL-1 β release, and behavioral effects, which we address in further detail below. Clearly, the studies by Alcocer-Gomez *et al.* (2015) and Iwata

et al. (2015) provide strong evidence for a causal role for the NLRP3 inflammasome in the effects of stress on depressive-like behavior.

It is remarkable that exposure to an acute or chronic stressor typically results in the induction of neuroinflammation in the absence of infection or pathogen exposure. Thus, consistent with previous work establishing sterile inflammation in the periphery after stressor exposure, these current studies suggest that stressor exposure may also stimulate sterile inflammation in the brain, or neuroinflammation (Chen and Nunez, 2010). As discussed previously, sterile inflammation is driven in large part by DAMPs that are released after physical and psychological stressors (Leemans *et al.*, 2011). Thus, it is reasonable to propose that sterile inflammatory processes have a mediating role in the effects of stress on inflammasome function, neuroinflammation, and the behavioral sequelae of inflammatory processes. Of note, there is some evidence that neuronal damage may occur in major depression (Duman, 2009), suggesting that DAMPs may also have a role in the pathophysiology of depression. Here, we explore several studies that address this notion.

Mechanisms of Stress and NLRP3 Inflammasome Priming/Activation

HMGB1

The DAMP HMGB1 has been implicated in stress-induced priming of NLRP3 and the neuroinflammatory response to a subsequent immune challenge (Frank *et al.*, 2015b). HMGB1 has a pivotal role in the induction of neuroinflammatory processes in neurodegenerative conditions (Fang *et al.*, 2012), cerebral ischemia (Yang *et al.*, 2010), traumatic brain injury (Corps *et al.*, 2015), seizure (Vezzani, 2014), and ethanol-induced neurotoxicity (Zou and Crews, 2014). HMGB1 is a DNA structural protein that has been co-opted by the innate immune system to signal damage during sterile inflammatory conditions (Bianchi, 2007). For example, cellular necrosis is a hallmark of several neuroinflammatory conditions. Here, HMGB1 is passively released into the extracellular space where it signals through TLRs (eg, TLR2/TLR4) to induce NF- κ B signaling and proinflammatory cytokine production. HMGB1 can also function as a chemotactic factor and also be actively secreted from innate immune cells (Yang *et al.*, 2013). Of particular relevance here, HMGB1 has been found to prime the NLRP3 inflammasome (Xiang *et al.*, 2011).

We recently found that exposure to an acute stressor (inescapable tailshock) induces HMGB1 in the hippocampus concomitant with increased protein levels of active NF- κ B and NLRP3 protein (Weber *et al.*, 2015). A number of studies have demonstrated that prior exposure to acute or chronic stressors potentiates the neuroinflammatory and microglial proinflammatory response to a subsequent immune challenge (Frank *et al.*, 2015b). Interestingly, we found that intracisterna magna administration of an HMGB1 antagonist (box A) before stress exposure blocked the potentiated

microglial proinflammatory response to an immune challenge *ex vivo*. This finding suggests that HMGB1 mediates stress-induced priming of the microglia proinflammatory response.

Consistent with these findings, Cheng *et al.* (2016) also found that exposure to a series of footshocks increased hippocampal protein levels of HMGB1 along with increased active NF- κ B, NLRP3, and mature caspase-1 protein (Cheng *et al.*, 2016). As noted earlier, initial stress exposure failed to increase IL-1 β protein; however, exposure to second series of footshocks 24 h after the initial series of footshocks resulted in increased hippocampal IL-1 β protein and mRNA. Intranasal pre-treatment with HMGB1 siRNA was found to significantly reduce hippocampal protein levels of HMGB1 and blocked stress-induced increases in IL-1 β protein, suggesting that HMGB1 mediates the priming effects of stress on IL-1 β . Further, Cheng *et al.* (2016) found that the effects of stress on NF- κ B, NLRP3, and mature caspase-1 were also blocked in TLR4 KO mice. However, stress effects on HMGB1 were not blocked in TLR4 KO mice.

This set of findings suggests that initial stress exposure induces the release of HMGB1 in the CNS, which then signals through TLR4 to prime the NLRP3 inflammasome. Upon exposure to a subsequent series of footshocks, the IL-1 β response was potentiated. Again, a key question here is what signal mediates NLRP3 inflammasome activation upon exposure to a second series of footshocks. It should be noted that exposure to the second series of footshocks resulted in a potentiated HMGB1 response in the hippocampus, suggesting that HMGB1 may serve as both the NLRP3 inflammasome priming stimulus as well as the activating stimulus.

We recently characterized the importance of the redox state of HMGB1 in the priming of the neuroinflammatory and depressive behavioral responses to a subsequent immune challenge (Frank *et al.*, 2015a). HMGB1 can function either as a chemotactic or proinflammatory mediator depending on the redox state of three critical cysteines (Venereau *et al.*, 2012). The disulfide form mediates the proinflammatory effects of HMGB1 by signaling through TLR4, whereas the reduced form mediates the chemotactic properties of HMGB1 by forming a complex with the chemokine CXCL12 and signaling through CXCR4. We found that the disulfide form upregulates NLRP3 expression and potentiates the neuroinflammatory response to a peripheral LPS challenge. In addition, we found that the disulfide form potentiated the LPS-induced reductions in social exploration as measured in the juvenile social investigation test.

Taken together, these findings suggest that HMGB1 mediates stress-induced priming of the neuroinflammatory and behavioral response to subsequent immune challenges and that HMGB1 priming of the NLRP3 inflammasome likely mediates these stress effects on neuroinflammation and behavior.

ATP

ATP is also considered to be a danger signal that is released from stressed or injured cells to signal damage to innate

immune cells (Ferrari *et al.*, 2006). Iwata *et al.* (2015) have found that exposure to acute immobilization stress (60 min) resulted in the intrahippocampal release of ATP concomitant with IL-1 β protein as well as NLRP3 inflammasome activation. These effects of stress were prevented in a *Nlrp3* KO mouse and were blocked by peripheral administration of an antagonist to the P2X7 receptor, which mediates ATP activation of the NLRP3 inflammasome (Jin and Flavell, 2010), suggesting that stress-induced release of ATP mediates NLRP3 inflammasome activation and subsequent release of IL-1 β . Further, they found that chronic administration of a P2X7 receptor antagonist during exposure to CUS ameliorated several effects of stress on depressive-like behaviors including the sucrose preference test, the novelty suppressed feeding test, and elevated plus maze. Although the role of ATP and P2X7 in this model is clear, it is not possible to conclude that this signaling is restricted to the brain because both the P2X7 receptor antagonist and the *Nlrp3* KO could have impacted sterile inflammation in the periphery as well as the brain (Karmakar *et al.*, 2016).

Importantly, a key question that remains with regard to stress-induced DAMP signaling and neuroinflammation is the mechanism whereby stress exposure induces DAMP release in the CNS. A number of mediators of the stress response, most notably noradrenaline and glucocorticoids, have been found to mediate the effects of stress on neuroinflammatory processes (Frank *et al.*, 2013; Wohleb *et al.*, 2011). However, it is unknown whether these stress mediators induce DAMPs, which may then function as the proximal signals of neuroinflammation.

Future Directions, Clinical Implications, and Potential Therapeutics

It is becoming increasingly accepted that inflammation contributes to an array of CNS disorders including MDD (Howren *et al.*, 2009; Schiepers *et al.*, 2005). In addition, stress is a well-established trigger of affective dysfunction and severe and/or repeated psychological stressor exposure also increases inflammatory proteins in blood and tissues. Growing evidence supports the hypothesis that sterile inflammatory processes likely have a role in stress-induced inflammation (Figure 4). Thus, understanding the mechanisms of sterile inflammation, the unique DAMP/MAMP/PAMP signaling in the periphery and CNS, and the role of inflammasome activation is critical for advancing the development of novel therapeutics to be used in the treatment of affective disorders (Miller *et al.*, 2015). In fact, there are several promising pharmacological candidates in use or in development that specifically target the inflammasome for the treatment of CNS inflammation after brain injury and stroke (de Rivero Vaccari *et al.*, 2016a, b). Neutralizing antibodies targeting NLRP1 was reported to reduce CNS inflammation after stroke in mice (Abulafia *et al.*, 2009). In addition, de Rivero Vaccari *et al.*, 2008, 2016b reported that antibody neutralization of ASC reduced CNS

Figure 4. A model of stress-induced sterile inflammatory processes in the brain and the neural impact of these processes. We propose that exposure to stressors results in the release of danger-associated molecular patterns (DAMPs) within the brain, presumably from damaged or dying neurons. These neuron-derived DAMPs then target their cognate receptors on microglia leading to inflammasome activation and the synthesis and secretion of interleukin-1 β (IL-1 β). The secreted form of IL-1 β may drive the induction of indoleamine 2,3-dioxygenase (IDO), which catabolizes tryptophan into kynurenine and thereby reduces the available pool of tryptophan for serotonin (5-HT) synthesis. Reductions in 5-HT synthesis may then mediate, in part, the effects of stress on sickness behavior.

inflammasome activation and inflammation after neural trauma. Although not yet tested in psychiatric patients, these advances could aid the rapid development of therapeutics for MDD.

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