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Stress-induced neuroinflammatory priming: A liability factor in the etiology of psychiatric disorders

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ABSTRACT

Stress and glucocorticoids (GCs) have universally been considered to be anti-inflammatory, however in recent years, stress and GCs have been found to exert permissive effects (immunological priming) on neuroinflammatory processes. This phenomenon of priming is characterized by prior stress or GC exposure potentiating the neuroinflammatory response to a subsequent immune challenge. A considerable body of evidence is discussed here that supports this permissive effect of stress and GCs.

In light of this evidence, a mechanism of neuroinflammatory priming is proposed involving a signal cascade in the brain involving danger-associated molecular patterns (HMGB-1) and inflammasomes (NLRP3), which results in an exaggerated or amplified neuroinflammatory response and subsequently, the amplification of the physiological and behavioral sequelae of this response (i.e. sickness). Finally, we explore the notion that stressor-induced sensitization of the neuroimmune microenvironment may predispose individuals to psychiatric disorders, in which exaggerated innate immune/inflammatory responses in the brain are now thought to play a key role.

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1. Introduction

Exaggerated innate immune/inflammatory responses in the brain are now thought to be a common core in the etiology of numerous psychiatric disorders such as depression, PTSD and bipolar disorder (Jones and Thomsen, 2013). Seemingly unrelated to neuroinflammation, the experience of life stressors is a predisposing factor in the development of psychiatric disorders (Kessler, 1997). However, the clinical literature contains numerous instances in which stress predisposes individuals to inflammatory disorders such as cardiovascular disease (Albus, 2010), which have a high comorbidity with psychiatric conditions that include depression (Sansone and Sansone, 2008). Indeed, recent evidence from several laboratories, including our own, suggests that stressors sensitize or prime the neuroinflammatory response to subsequent pro-inflammatory challenges, thereby providing a mechanism that can link the impact of stressors and a pivotal role for neuroinflammatory processes in the development of psychiatric disorders.

The central notion explored here is that exposure to stressors can induce a vulnerable phenotype characterized, in part, by a sensitized neuroimmune microenvironment. Thus, prior stress can lead to a potentiated neuroinflammatory cascade upon exposure to pro-inflammatory challenges that include bacterial or viral infection and sterile injury. This exaggerated neuroinflammatory response can then manifest as changes in cognitive (e.g. memory), affective (e.g. mood), sensory (e.g. pain) and vegetative (e.g. sleep and eating) endophenotypes, typically observed in a spectrum of psychiatric disorders. Several neuroimmune mechanisms, by which stress sensitizes/primes the neuroinflammatory response to pro-inflammatory challenges, have been characterized in recent years. First, a brief overview of neuroinflammatory processes will be provided as a basis for exploring mechanisms of neuroinflammatory priming.

2. Neuroinflammatory processes and microglia

Neuroinflammatory processes are characterized, in large part, by the activation of CNS innate immune effector cells including microglia, which secrete an array of inflammatory mediators including pro-inflammatory cytokines, chemokines, prostaglandins and reactive oxygen/nitrogen species (Ransohoff and Perry, 2009). When elaborated under a variety of neuropathological conditions, including neurodegeneration, brain trauma, ischemia and seizure, these mediators induce the hallmarks of neuroinflammation, which may include recruitment of peripheral leukocytes into the CNS (Graeber et al., 2011). Of note, peripheral infection or immune challenge also is capable of inducing a neuroinflammatory response in the brain (McCusker and Kelley, 2013). For example, peripheral injection of lipopolysaccharide (LPS), a non-infectious component of the cell wall of gram-negative bacteria (e.g. *E. coli*), is commonly used as an immunogenic stimulus to induce a pro-inflammatory response in peripheral biological compartments or organs such as liver, spleen, serum or peritoneum. As a result of pathogen exposure or immune challenge with agents such as LPS, peripheral myeloid cells, such as splenic macrophages or liver Kupffer cells, secrete pro-inflammatory cytokines, which then signal the brain through several well-characterized neural and humoral pathways connecting the immune system with the CNS (Maier, 2003). A neural cascade initiated by immune-to-brain signaling then leads to the production of pro-inflammatory cytokines and other inflammatory products by microglia and other CNS innate immune cells as if these cells had been directly exposed to the pathogenic agent (McCusker and Kelley, 2013). It should be noted that peripheral pathogens are typically excluded from entering the brain and directly activating CNS innate immune cells by the blood brain barrier (Banks, 2015). Thus, cytokine mediated immune-to-brain signaling is a pivotal immunologic process whereby the immune system communicates to the brain that infection or injury has occurred in the periphery. Once the CNS is alerted of an immunologic threat to the organism, the subsequent neuroinflammatory response engages neural mechanisms that mediate physiological and behavioral modifications, also known as the sickness response, which are key to effective host defense (Dantzer et al., 2008). Interestingly, aspects of the sickness response resemble aspects of several mood disorders, most notably major depression (Dantzer, 2009), a theme we review in section 3.

2.1. Microglia function and activation states

As noted above, microglia are key effectors of neuroinflammatory processes and thus here we will briefly describe the physiological aspects of microglia function that are relevant for understanding the role of this CNS innate immune cell in stress-induced neuroinflammatory priming. 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, microglia are maintained in the adult CNS independent of circulating blood monocytes and are thought to self-renew from progenitor cells in the CNS (Katsumoto et al., 2014). 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), which are characterized by varying blends of immunophenotypes and cytokine profiles. Of particular relevance here, a primed activation state may be induced in microglia under several neuroinflammatory conditions (Perry et al., 2007) that include exposure to psychological stressors and stress hormones such as glucocorticoids (Frank et al., 2015a). Microglia are considered primed if, upon exposure to a pro-inflammatory stimulus such as LPS, the pro-inflammatory cytokine response of microglia is potentiated beyond normal. For example, we have found that prior exposure to a severe acute stressor potentiates the pro-inflammatory response of microglia to a subsequent immune challenge (i.e. LPS) *ex vivo* (Frank et al., 2007). A primed state has been associated with morphological changes such as shrunken processes and increased soma size, as well as immunophenotypic changes such as increased expression of surface antigens including major histocompatibility class II (MHCII) (Norden et al., 2015). Importantly, a primed activation state may not involve increased pro-inflammatory output. However, it is unclear whether a primed activation state involves uniform changes in microglia morphology and immunophenotype. This primed state of activation can be conceptualized as a ‘readiness’ state because, when in this state, microglia will rapidly respond to inflammatory stimuli with exaggerated production of pro-inflammatory cytokines.

2.2. Toll-like receptors (TLRs)

Microglia express an array of receptors that allow microglia to rapidly mount an immune response to exogenous or endogenous immunological threats (Rivest, 2009). At least 50 cell surface antigens distinguish mononuclear phagocytes from other cell types

(Ransohoff and Cardona, 2010). Of these, TLRs will be a focus as they are thought to play a pivotal role in stress-induced neuroinflammatory priming (Frank et al., 2015b). Microglia express many of the TLRs characterized in peripheral immune cells (Carpentier et al., 2008). Cells of the innate immune system recognize microbial products via germ line-encoded receptors that recognize general molecular patterns or motifs that characterize classes of pathogens (Pathogen Associated Molecular Patterns; PAMPs (Janeway and Medzhitov, 2002)). For this reason, receptors that recognize PAMPs have been termed pattern recognition receptors (PRRs). Of these PRRs, TLRs are the most extensively characterized (Barton and Kagan, 2009). TLRs are a family of highly conserved membrane or cytosolic (dependent on TLR subtype) proteins that transduce signals through a family of cytosolic Toll adapter proteins that link to downstream signaling cascades (Barton and Kagan, 2009). The ligation of many of the TLR family members ultimately activates the transcription factor, nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB), as well as other transcription factors (Salminen et al., 2008), and NF-κB activation is viewed as key to the inflammatory effects of TLR ligation (Salminen et al., 2008). TLR2 and TLR4 have been the most intensively studied PRRs within the CNS, and are expressed on microglia (Aravalli et al., 2007). They are sometimes reported to be present on astrocytes and sometimes absent, and are typically reported to be absent on neurons (Lehnardt et al., 2003). TLR4 recognizes the LPS motif present in the cell membrane of gram-negative bacteria, while TLR2 recognizes lipoteichoic acid that characterizes gram-positive bacteria (Kawai and Akira, 2007).

Although TLRs were first studied as receptors for microbial products that then activate immune cells such as macrophages and dendritic cells, thereby initiating host defense against infection, TLRs have also been found to recognize endogenous molecular patterns. Over the past several decades it has become clear that peripheral immune responses are initiated by a number of conditions that do not involve pathogen entry into the body such as a sterile bone break (Chen and Nunez, 2010). These conditions have been summarized as “danger” (Bianchi and Manfredi, 2009), and the necessary implication is that these dangers must lead to the release of *endogenous* molecules that act to initiate innate immune responding. A number of these molecules have been characterized and have been collectively called danger-associated molecular patterns (DAMPs) or alarmins (Bianchi, 2007). Interestingly, one of these DAMPs, high mobility group box 1 protein (HMGB-1) is recognized by TLR2 and TLR4 (Yang et al., 2013) and is present in brain (Fang et al., 2012). HMGB-1 is thought to alert innate immune cells to a variety of internal conditions such as cellular stress, damage or necrosis (Kawai and Akira, 2010). Of note, TLR2 and TLR4 recognize other DAMPs including histones, heat shock proteins and S100 proteins (Venereau et al., 2015), which may play a role in neuroinflammatory priming. However, HMGB1 is of particular interest here because of its direct role in priming of inflammasome signaling (See section 2.4.)

2.3. HMGB-1 and TLR signaling

Our interest has centered on the DAMP HMGB-1, which stemmed from our initial finding that pharmacologic blockade of TLR2 and TLR4 during stress exposure abrogates stress-induced priming of the neuroinflammatory response to a subsequent immune challenge (Weber et al., 2013) (see Section 4.2.). HMGB-1 is a ubiquitous nuclear DNA binding protein, which under normal conditions, is not present in the extracellular space (Kang et al., 2014). However, under necrotic conditions, HMGB-1 is passively released into the extracellular milieu to serve as an inflammatory signal (Scaffidi et al., 2002). As noted above, TLR2 and TLR4 mediate the pro-inflammatory effects of HMGB-1, although some recent studies now suggest that the pro-inflammatory effects of HMGB-1 are mediated predominately through TLR4 (Yang et al., 2013). The receptor for advanced glycation end products (RAGE) and the chemokine receptor CXCR4 are thought to mediate the chemotactic function of HMGB-1 (Yang et al., 2013). Interestingly, immunocompetent cells also have the unique ability to actively release HMGB-1 in the absence of cell death (Bonaldi et al., 2003).

There are three distinct forms of HMGB-1 characterized by post-transcriptional modification of the redox state of three critical cysteine residues (C23, C45, and C106). Fully reduced (fr) HMGB-1 is the predominant form that occurs under non-oxidizing conditions. fr-HMGB-1 has chemotactic properties produced by the formation of a hetero-complex with the chemokine CXCL12, which then signals through the chemokine receptor CXCR4 (Schiraldi et al., 2012). The pro-inflammatory form occurs under increased oxidizing conditions, in which a disulfide bridge forms between C23 and C45 (disulfide (ds) HMGB-1). This redox form interacts with TLR4 to induce synthesis and secretion of pro-inflammatory cytokines (Antoine et al., 2014). Finally, the fully oxidized form of HMGB-1 has no known biological activity (Venereau et al., 2012).

2.4. TLR4, the NLRP3 inflammasome and IL-1β

The formation and secretion of IL-1β is a critical step in the neuroinflammatory cascade and IL-1β has been termed the “master regulator” of neuroinflammation (Basu et al., 2004), given its pleiotropic role in the induction of central inflammatory processes as well as its critical role in the manifestation of the sickness response (Goshen and Yirmiya, 2009). Thus, the production, processing and signaling of IL-1β is under tight regulation (Dinarello, 2011). IL-1β is transcribed as a larger pro-hormone, pro-IL-1β, and pro-IL-1β must be cleaved by caspase-1 to form the biologically active, mature form of IL-1β.

The process of cleaving pro-IL-1β typically requires the formation and activation of an inflammasome, most often the nucleotide-binding domain and leucine-rich repeat containing family, pyrin domain containing 3 (NLRP3) inflammasome, an intracellular multiprotein complex that mediates processing and maturation of IL-1β via activation of caspase-1 (Martinon et al., 2009). Importantly, signaling at TLR4 initiates an intracellular signaling cascade to activate the immune-related transcription factor NF-κB (Kawai and Akira, 2010), which is a critical step in the formation of the NLRP3 inflammasome. The NLRP3 inflammasome is particularly interesting because of 1) its unique functional requirements that include a priming step and an activation step (Hornung and Latz, 2010) and 2) the role of glucocorticoids in priming of the NLRP3 inflammasome (Busillo and Cidlowski, 2013) (see section 4.4.). Priming occurs in response to a stimulus that signals, in part, through TLR4 to increase the expression of the sensor protein NLRP3 to a critical threshold (Latz et al., 2013) (Fig. 1). Interestingly, HMGB-1 has been reported to increase NLRP3 mRNA and protein (Xiang et al., 2011) suggesting that HMGB-1 may function as a priming stimulus of the NLRP3 inflammasome. Subsequently, a second activating signal such as adenosine triphosphate (ATP) is then required to form the NLRP3 inflammasome complex with the adapter protein ASC, which then recruits and cleaves pro-caspase-1 into mature caspase-1 (Latz et al., 2013). In turn, caspase-1 cleaves pro-IL-1β into mature IL-1β. It is to be noted that other pro-inflammatory cytokines, such as IL-6 and TNFα, are not under this type of tight regulation and are not inflammasome dependent (Kumar et al., 2011).

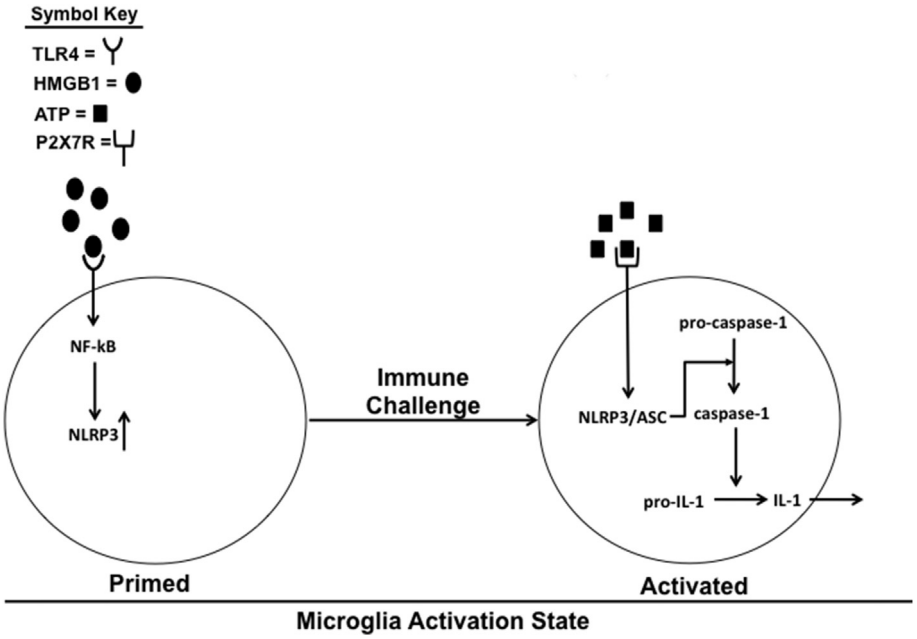

Fig. 1. Priming and activation of the NLRP3 inflammasome. The canonical pathway of NLRP3 inflammasome activation is thought to require a two-step process. An initial priming step involves signaling through pattern recognition receptors such as TLR4 and subsequent activation of NF-kB, which then drives transcription of NLRP3. Once NLRP3 mRNA/protein is increased to a critical threshold, the cell is considered primed, but pro-inflammatory cytokine output is not increased. Upon exposure to a second activating signal such as ATP, NLRP3 forms a complex with ASC. This complex recruits pro-caspase-1, which is then converted to mature caspase-1. Caspase-1 is the rate-limiting enzyme in the conversion of pro-IL-1β to mature IL-1β. Once formed, mature IL-1β is then released via an unconventional pathway independent of the Golgi apparatus. Abbreviations: HMGB-1 (high mobility group box-1); NLRP3 (nucleotide-binding domain and leucine-rich repeat containing family, pyrin domain containing 3); TLR4 (Toll-like receptor 4); NF-κB (nuclear factor of kappa light polypeptide gene enhancer in B-cells); ATP (adenosine triphosphate); P2X7R (purinergic receptor P2X, ligand gated ion channel, 7); ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain); IL (interleukin).

3. Cytokines, sickness behavior and mood disorders

While pro-inflammatory cytokines are key mediators subserving critical immune functions of microglia and other CNS innate immune cells, these mediators of neuroinflammation also orchestrate a constellation of physiological and behavioral modifications known as the sickness response (Dantzer et al., 2008). Beyond the scope of the present review, IL-1β is a particularly important mediator of the sickness response and has been the subject of several in-depth reviews (for example see review by (Goshen and Yirmiya, 2009)). However, it should be noted that other pro-inflammatory cytokines including TNFα play a role in the sickness response to pro-inflammatory stimuli (Dantzer et al., 2008). This response includes cognitive (memory), affective (mood), vegetative (sleep and eating), sensory (pain) and physiological (fever) changes, which play an adaptive role in an organism's host defense against infection, trauma, and injury (Dantzer, 2009). Of note, there is a remarkable similarity between the hallmarks of the sickness response and clinical endophenotypes typically observed in several psychiatric disorders including major depression and anxiety disorders. For example, anhedonia is a symptom typically observed in major depression and is also a hallmark of the sickness response to infection or injury (Dantzer et al., 2008). Indeed, a considerable number of findings suggest that inflammatory processes may play a role in the etiology of major depression (Raison and Miller, 2013). Moreover, some of the strongest evidence implicating pro-inflammatory cytokines in the pathogenesis of depression stems from studies demonstrating that treatment with pro-inflammatory agents is sufficient to induce depressive symptoms (Miller et al., 2009). Of particular relevance here, prior exposure to acute and chronic stressors potentiates the sickness response to subsequent pro-inflammatory challenges. Further, prior exposure to stress induces a primed microglial immunophenotype such that the neuroinflammatory response to a subsequent inflammatory challenge is also potentiated (See section 4.2.). Therefore, we propose that stress-induced priming of neuroinflammatory processes may serve to predispose individuals to a heightened neuroinflammatory response as well as the neural/behavioral sequelae of that response if exposed to a subsequent pro-inflammatory insult. In doing so, this neuroinflammatory priming effect of stress may function as an etiological risk factor in the development of major depression or other psychiatric disorders in which inflammatory processes have been implicated in disease pathogenesis (Jones and Thomsen, 2013).

4. Mechanisms of neuroinflammatory priming

4.1. Acute inflammatory responses to a stressor

Exposure to an acute stressor induces a rapid and brief (2–6 h) increase of pro-inflammatory cytokines in stress-reactive areas of the brain, including the hypothalamus and hippocampus (Johnson et al., 2005). As a result, acute exposure to a stressor also induces physiological and behavioral sickness response changes that are similar to those produced by a peripheral immune challenge. A stressor as seemingly minor as placing a rat in a novel environment produces fever and impairs contextual fear conditioning (LeMay et al., 1990; Pugh et al., 1999). Other typical sickness responses have also been observed after stress exposure, including decreased body weight, food and saccharine consumption, and social exploration (Christianson et al., 2008; Johnson et al., 2003). It is known that these sickness responses result from the cytokine increases because blockade of cytokine receptors in the brain and/or cytokine synthesis blocks or reduces changes in these responses (Goshen and Yirmiya, 2009). The rapid increase in the pro-inflammatory cytokine and behavioral response to a stressor is due, at least in

part, to activation of noradrenergic processes in the brain and the associated release of catecholamines (Johnson et al., 2005). Norepinephrine is a fundamental component of the fight/flight response that modulates physiological responses to a stressful event. Another major element of norepinephrine is to relay information to the immune system. In the CNS, stimulation of beta-adrenergic receptors increases IL-1β mRNA expression in glial cells (Johnson et al., 2005; Maruta et al., 1997). Moreover, peripheral administration of beta-adrenergic receptor antagonists prior to stress prevents subsequent brain cytokine responses and the related behaviors controlled by these cytokines (Blandino et al., 2009; Hanke et al., 2012; Johnson et al., 2005; Wohleb et al., 2011).

4.2. Neuroinflammatory priming

Exposure to a stressor not only produces a transient neuroinflammatory response, but also alters how an organism responds to a subsequent inflammatory challenge for a period of time *after* the stress exposure has ended. Several studies have found that prior exposure to a potent acute or chronic stressor potentiates the neuroinflammatory and microglial pro-inflammatory response, as well as the sickness response, to a subsequent immune challenge (de Pablos et al., 2014; de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Frank et al., 2007, 2012; Johnson et al., 2002, 2003, 2004; Munhoz et al., 2006; Weber et al., 2013, 2015; Wohleb et al., 2012; Wohleb et al., 2011). Importantly, in these studies, neuroinflammatory and microglial priming was induced using several types of stressors including chronic social defeat, chronic unpredictable mild stress and acute inescapable tail shock. The paradigm we employ involves 100 brief tailshocks that the subject, typically a rat, cannot escape (inescapable tailshock; IS). Control rats are left undisturbed in their home cage (home cage controls; HCC). 24 h after IS, cytokine levels in the brain are at control levels (Johnson et al., 2003). However, when an immunogenic agent such as LPS is administered, the pro-inflammatory cytokine response (IL-1β, IL-6, TNFα) in several brain regions (hypothalamus, hippocampus, and cortex) is potently exaggerated compared with HCC subjects that were administered the same immune challenge. As a result, physiological and behavioral processes that are controlled by these brain cytokines are also changed. For example, LPS-induced fever, corticosterone, adrenocorticotropic hormone, and decreased locomotor activity are all potentiated by prior exposure to IS (Johnson et al., 2003). It should be noted that the potentiated neuroinflammatory response to an immune challenge typically lasts 4–6 days after IS exposure (Johnson et al., 2002). This period of neuroinflammatory sensitization can be conceptualized as a period of vulnerability during which an organism is susceptible to the production of heightened neuroinflammatory processes, if exposed to an immune challenge. It should also be noted that the duration of this period of vulnerability is likely influenced by several factors, including the nature, intensity and duration of the stressor. In humans, understanding how stress predisposes individuals to develop psychiatric disorders is a key question that has only been partially answered. Given the link between psychiatric disorders and neuroinflammatory processes, our laboratory has been interested in understanding the central mechanism(s) by which exposure to a stressor impacts the neuroinflammatory environment.

4.3. Role of microglia

To begin to explore this question, we first examined the primary innate immune cell in the brain, microglia. One limitation of measuring cytokines in brain tissue is that the cellular source is unknown, as microglia, astrocytes, endothelial cells, neurons as well as other myeloid cells produce cytokines (Basu et al., 2004). In order to address this issue, our laboratory developed a procedure to isolate and study microglia *ex vivo* (Frank et al., 2006). As noted above, quiescent or surveillant microglia express low levels of the cell surface antigen MHCII, which is rapidly upregulated when microglia are activated (Aloisi, 2001) and is indicative of a primed activation state (Perry, 2004). Exposure to IS increased both mRNA and protein expression of MHCII on hippocampal microglia for at least 24 h after termination of the stressor. However, exposure to IS alone had no effect on hippocampal IL-1β mRNA levels 24 h later (Frank et al., 2007). That is, although MHCII was upregulated on microglia, the cells were not producing IL-1β, suggesting that exposure to a stressor induces a primed state in microglia. Indeed, when microglia were isolated from rats that were exposed to IS and stimulated with LPS *ex vivo*, the pro-inflammatory cytokine response was significantly increased compared with HCC microglia stimulated with LPS (Frank et al., 2007). A study by Wohleb and colleagues reported similar findings using repeated social defeat as a stressor (Wohleb et al., 2011). Mice were given 6 consecutive days of social defeat stress and whole brain microglia were isolated and treated with LPS *ex vivo*. Prior exposure to social defeat potentiated the microglial pro-inflammatory response to LPS compared to the pro-inflammatory response of microglia isolated from HCC mice. Taken together, these findings suggest that acute and chronic stressors prime microglia such that exposure of microglia to a subsequent immune challenge results in an exaggerated pro-inflammatory response. Further, these findings imply that stressors may induce the release of a signaling molecule in the brain that modulates the immunophenotype of microglia, thereby inducing a primed activation state. Towards addressing this possibility, we focused on the role of stress-induced glucocorticoids (GCs), which on the face of it seems counter-intuitive given the preponderance of evidence showing that GCs are largely anti-inflammatory (Boumpas et al., 1993). But, as we detail in section 4.4, GCs have also been found to have paradoxical effects, which are inconsistent with the anti-inflammatory properties typically attributed to GCs.

4.4. Stress, GCs and neuroinflammatory priming

Stress (Webster Marketon and Glaser, 2008) and GCs (Boumpas et al., 1993) have been historically regarded to be anti-inflammatory, and this concept has been a bedrock principle until very recently. It has never been clear how inhibition of innate immune inflammatory responses by GCs would be adaptive during a fight/flight emergency as these are periods of increased risk for infection and injury. This “conundrum” has provoked numerous theoretical attempts at a resolution (Frank et al., 2013; Munck et al., 1984). Moreover, a fair number of clinical trials have reported that GC therapy is not always anti-inflammatory and sometimes produces paradoxical results with the relevant condition being exacerbated rather than alleviated (Rutgeerts, 2001). Current thinking about the role of stress in the development of psychiatric disorders has also likely been constrained by the notion that stress must be anti-inflammatory, rather than pro-inflammatory. However, recent studies from our laboratory as well as several others now suggest that stress-induced GCs can actually promote or prime neuroinflammatory processes to subsequent immune challenges. Beyond the scope of the present review, it is important to note that a considerable number of studies have found that stress-induced noradrenergic processes as well as modulation of immune cell trafficking to the brain play a role in stress-induced neuroinflammatory priming (Wohleb et al., 2014), which may play a role in the processes of neuroimmune priming under discussion here.

A number of studies have found that pharmacological blockade of GC signaling during acute or chronic stress exposure ameliorates

the potentiated neuroinflammatory response to a subsequent immune challenge (de Pablos et al., 2014; de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Frank et al., 2012; Munhoz et al., 2006). Moreover, we found that pharmacological (RU486) and surgical (adrenalectomy) blockade of GC signaling during stress exposure abrogated the stress-induced potentiation of the microglia pro-inflammatory response to an immune challenge (LPS) *ex vivo* (Frank et al., 2012). These findings suggest that the stress-induced rise in GCs leads to a shift in the activation state of microglia from surveillant to primed. While these studies demonstrated the necessity of GC signaling in stress-induced neuroinflammatory priming (de Pablos et al., 2014; de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Frank et al., 2012; Munhoz et al., 2006), a number of studies have also found that exogenous GC treatment is sufficient to prime neuroinflammatory processes as well as the microglial pro-inflammatory response *ex vivo*.

We found that acute administration of corticosterone (CORT) potentiated the neuroinflammatory response to LPS that was administered 24 h after CORT injection (Frank et al., 2010). Moreover, we found that hippocampal microglia isolated 24 h after CORT injection and challenged with LPS *ex vivo* also exhibited a potentiated pro-inflammatory response to LPS. Interestingly, if CORT was administered 1 h after LPS, the neuroinflammatory response to LPS was suppressed. It should be noted that the dose of CORT employed in this study was found to induce a pattern in serum CORT concentrations that mimics that produced by inescapable tailshock (Fleshner et al., 1995). Subsequent studies have also found that chronic CORT administration primes the neuroinflammatory response as well as the *ex vivo* microglial pro-inflammatory response to a subsequent immune challenge (Frank et al., 2014; Munhoz et al., 2010). Although the mechanism(s) by which CORT primes microglia is still under investigation, Munhoz and colleagues found that prior CORT exposure potentiates the LPS induction of several signal transduction pathways including the NF-κB pathway (Munhoz et al., 2010). Similarly, we have found that chronic CORT treatment dose dependently increased NLRP3 gene expression as well as NF-κB inhibitor α, which is induced by NF-κB activation (Sun et al., 1993). Taken together, these studies suggest the possibility that GCs activate the NF-κB pathway, thereby leading to up-regulation of NLRP3 expression and priming of the NLRP3 inflammasome. Indeed, Busillo and colleagues have found that GCs increase NLRP3 expression in macrophage cell lines as well as potentiate the macrophage pro-inflammatory response to a subsequent immune challenge (Busillo et al., 2011). We have recently replicated this effect of GCs on NLRP3 expression in primary microglia (unpublished observations). However, it is unclear from these findings how GCs could directly induce NLRP3 or for that matter activate NF-κB. A likely possibility is that GCs prime neuroinflammatory processes via induction/release of danger signals, such as HMGB-1, in the brain. HMGB-1 may then target TLR4 on microglia to activate NF-κB and increase NLRP3 expression, thereby priming the NLRP3 inflammasome. To begin exploring this notion, we examined the role of HMGB-1 in neuroinflammatory priming.

4.5. HMGB-1 as a neuroinflammatory priming signal

In order for microglia to be primed by stress, microglia must first receive a priming signal. Recent evidence implicates HMGB-1 as one such signal. As noted above, our interest in HMGB-1 stemmed from an initial investigation demonstrating that blockade of TLR2 and TLR4 during stress exposure abrogated stress-induced priming of microglia (Weber et al., 2013). Given that TLR2 and TLR4 mediate the pro-inflammatory effects of HMGB-1, we explored the possibility that stress-induced HMGB-1 may play a role in neuroinflammatory priming. An initial investigation revealed that IS increases HMGB-1 protein in the hippocampus (Weber et al., 2015). Further investigation indicated that hippocampal microglia, isolated immediately after stress exposure, secreted an increased amount of HMGB-1 compared with microglia from control animals. Importantly, cell viability did not differ between stress and control groups suggesting that HMGB-1 was actively secreted by microglia in the absence of cell death.

The finding that stress increased hippocampal HMGB-1 expression led us to explore whether HMGB-1 plays a causal role in stress-induced priming of neuroinflammatory responses. To explore whether HMGB-1 is necessary, a competitive HMGB-1 antagonist, boxA, was injected into the brain prior to stress exposure. The blockade of HMGB-1 signaling in the brain prevented the stress-induced priming of hippocampal microglia, that is, box A administered prior to IS eliminated the exaggerated cytokine response of microglia exposed to LPS *ex vivo* 24 h after IS (Weber et al., 2015).

A key question concerns which redox form of HMGB-1 might mediate stress-induced neuroinflammatory priming. Interestingly, central administration of dsHMGB-1, but not frHMGB-1, primed hippocampal microglia to LPS *ex vivo* (Weber et al., 2015). That is, dsHMGB-1 mimicked the effect of stress exposure on microglia and is likely the form that is released during stress exposure to prime microglia.

Another key issue concerns the mechanism by which dsHMGB-1 primes microglia. One major difference between the 2 active forms of HMGB-1 is their receptor targets. In this regard, dsHMGB-1 is a ligand for TLR4, while frHMGB-1 interacts with CXCR4 via CXCL12 (Yang et al., 2013). Signaling at TLR4 initiates an intracellular signaling cascade that activates the immune-related transcription factor NF-κB (Kawai and Akira, 2007) and priming of the NLRP3 inflammasome (Hornung and Latz, 2010), while CXCR4 activates a variety of signaling pathways including ERK1/2, p38, SAPK/JNK and mTOR, which are associated with calcium influx, cell mobility and survival (Ehtesham et al., 2013). Therefore, the ability of dsHMGB-1 to prime microglia likely occurs via the TLR4/NF-κB pathway.

Initially, we found that IS activates NF-κB and increases NLRP3 protein in the hippocampus, yet these changes were not associated with alterations in IL-1β protein levels (Weber et al., 2015). This finding would suggest that the NLRP3 inflammasome is 'primed' but not 'active' after exposure to the stressor. A similar effect was observed by Pan and colleagues in the prefrontal cortex after exposure to chronic mild stress (Pan et al., 2014). Moreover, NLRP3 inhibition during chronic mild stress prevented pro-inflammatory cytokine increases (Liu et al., 2015). As noted in section 2.4, HMGB-1 has been reported to increase NLRP3 mRNA and protein (Xiang et al., 2011) and it has been argued that danger signals such as HMGB-1 prime the NLRP3 inflammasome (Leemans et al., 2011). This argument is supported by our finding that pharmacological blockade of HMGB-1 signaling during stress exposure prevents NLRP3 sensitization in microglia (Weber et al., 2015). Given that NLRP3 priming can occur via TLR4 and TLR4 is a receptor target for dsHMGB-1, we hypothesize that stress-induced dsHMGB-1, but not frHMGB-1 primes the NLRP3 inflammasome. Thus, stressors, via induction of increased secretion of dsHMGB-1, appear to prime the inflammasome so that subsequent inflammatory challenges lead to exaggerated pro-inflammatory mediators in the brain.

5. Summary

The effect of stressors on GCs, HMGB-1 and the NLRP3 inflammasome provides a potential mechanism by which stress exposure primes neuroinflammatory processes (Fig. 2). Exposure to a stressor can be conceptualized as 'danger'. We propose that in

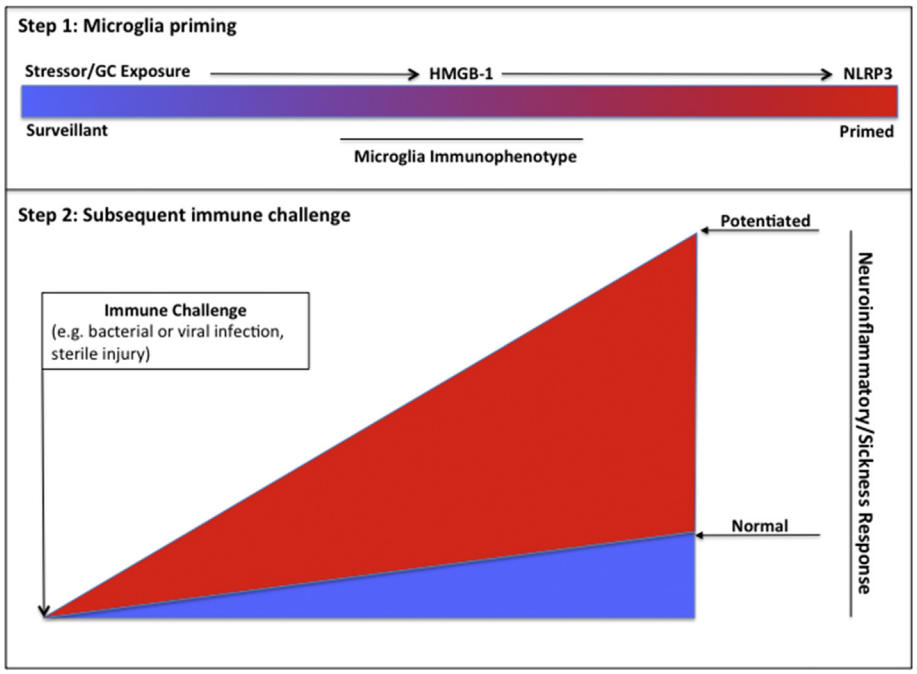

Fig. 2. A model of the neuroimmune mechanism of stress- and GC-induced amplification of the neuroinflammatory response to immune challenges. We propose that a 2-step process is required for stress/GC-induced potentiation of the neuroinflammatory response to a subsequent immune challenge. In the top panel (step 1), a mechanism of microglia priming is proposed. Upon stress/GC exposure, increased levels of central GCs induce the extra-cellular release of HMGB-1 in the brain. In turn, HMGB-1 induces the upregulation of NLRP3 expression in microglia and other CNS innate immune cells, most likely through the TLR4/NF-κB signaling pathway. With the upregulation of NLRP3, microglia are considered primed. In the bottom panel (step 2), if a subsequent immune challenge occurs while microglia are primed, the ensuing neuroinflammatory and sickness response to that challenge is potentiated compared to the "normal" neuroinflammatory response of surveillant microglia to the same immune challenge. Abbreviations: GC (glucocorticoid); HMGB-1 (high mobility group box-1); NLRP3 (nucleotide-binding domain and leucine-rich repeat containing family, pyrin domain containing 3); TLR4 (Toll-like receptor 4); NF-κB (nuclear factor of kappa light polypeptide gene enhancer in B-cells).

response to stressors, GCs induce the release of HMGB-1 in the brain, in part, by microglia. It should be noted that neurons are a significant source of HMGB-1 in the CNS (Fang et al., 2012) and thus may be involved in the processes under discussion. Once released into the extracellular space, HMGB-1, likely in the disulfide redox state, signals microglia in an autocine/paracrine manner via TLR4. Thus, binding at TLR4 activates NF-κB to synthesize NLRP3, and the cell is now considered primed. In the event of a subsequent immune challenge, formation of the NLRP3 inflammasome is potentiated, resulting in greater caspase-1 activation and cleavage of pro-IL-1β into the mature and bioactive IL-1β. IL-1β then initiates a cascade of neuroinflammatory processes including the release of other pro-inflammatory cytokines and inflammatory mediators. Therefore, the net result is an exaggerated or amplified neuroinflammatory response and subsequently, the physiological and behavioral sequelae of this cascade (i.e. sickness) are amplified.

6. Future perspectives

While prior stress exposure is considered a predisposing factor in the development of psychiatric disorders (Kessler, 1997), a key question remains: why does stress lead to the development of psychiatric disorders in some individuals, but not in others? A number of factors mediating the relationship between stress and psychiatric disorders have been proposed including individual differences in biological, developmental, psychological and sociodemographic factors (Hammen, 2005). Of these factors, sex differences are of particular relevance here insofar as females are more susceptible to developing affective psychiatric disorders as well as autoimmune disorders, which reflects the tendency of females to display enhanced immune responses (Schwarz and Bilbo, 2012). Moreover, Bilbo and colleagues have found that pro-inflammatory mediators are basally elevated in the brain of adult female rats compared to males. Moreover, microglia of adult females exhibit an activated phenotype (Schwarz et al., 2012). These findings suggest that females may be particularly vulnerable to stress-related psychiatric disorders due to the tendency for heightened neuroimmune responses.

The findings reviewed here provide a framework for understanding how exposure to stressors may function as an additional vulnerability factor in the development of psychiatric disorders, particularly in females and individuals with a predisposition towards a heightened neuroimmune response. If indeed exposure to stressors shifts the neuroimmune microenvironment towards a primed state, then a window of vulnerability to heightened neuroinflammatory processes is opened. If a pro-inflammatory challenge were to occur during this window, the heightened neuroinflammatory response may lead to dysregulation of the neuroimmune microenvironment, thereby increasing the likelihood of developing mood disorders. Pro-inflammatory challenges can take many forms such as the obvious, for example bacterial or viral infection, but also the less obvious, such as sterile injury, drugs of abuse and of course, additional stress exposure. Therefore, understanding the mechanisms of neuroinflammatory priming may lead to the development of therapeutics, which, if applied during this window of vulnerability, could be used to desensitize the neuroimmune microenvironment and prevent the development of mood disorders.

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