THE ROLE OF INFLAMMATION AND MICROGLIAL ACTIVATION IN THE PATHOPHYSIOLOGY OF PSYCHIATRIC DISORDERS


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Abstract—Psychiatric disorders, including major depressive disorder (MDD), bipolar disorder (BD) and schizophrenia, affect a significant percentage of the world population. These disorders are associated with educational difficulties, decreased productivity and reduced quality of life, but their underlying pathophysiological mechanisms are not fully elucidated. Recently, studies have suggested that psychiatric disorders could be considered as inflammatory disorders, even though the exact mechanisms underlying this association are not known. An increase in inflammatory response and oxidative stress may lead to inflammation, which in turn can stimulate microglia in the brain. Microglial activation is roused by the M1 phenotype, which is associated with an increase in interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). On the contrary, M2 phenotype is associated with a release of anti-inflammatory cytokines. Thus, it is possible that the inflammatory response from microglial activation can contribute to brain pathology, as well as influence treatment responses. This review will highlight the role of inflammation in the pathophysiology of psychiatric disorders, such as MDD, BD, schizophrenia, and autism. More specifically, the role of microglial activation and associated molecular cascades will also be discussed as a means by which these neuroinflammatory mechanisms take place, when appropriate.

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Key words: microglia, neuroinflammation, major depressive disorder, bipolar disorder, autism, schizophrenia.

Contents

Introduction 141
The role of microglia in stress and depression 142
The role of microglia in BD 146
Microglial hypothesis of schizophrenia 147
Microglial activation in autism 149
Conclusion 150
Acknowledgments 150
References 150

INTRODUCTION

A growing body of evidence suggests that many psychiatric disorders, including major depressive disorder (MDD), bipolar disorder (BD), schizophrenia, and autism are associated with distinct inflammatory mechanisms in the periphery and in the central nervous system (CNS). The relevance of inflammation in these conditions has been proposed by several studies, linking them with alterations in cytokines and acute-phase reactants. Risk factors for MDD and BD include medical conditions associated with chronic inflammatory and immunological alterations, such as rheumatoid arthritis, obesity and diabetes (Leboyer et al., 2012). Moreover, peripheral immune modulators have been shown to induce psychiatric symptoms in humans and in animal models (Dantzer et al., 2008; Harrison et al., 2009; Eisenberger et al., 2010; Haroon et al., 2012). Inflammation in the context of the nervous system, termed...
‘neuroinflammation’, has been reported in patients with psychiatric disorders (Najar et al., 2013), and is typically associated with microglial activation.

Microglia are CNS-resident cells that are usually the first to be activated in response to tissue damage or brain infections (Stertz et al., 2013). These small cells have several functions described, including (but not limited to): pathogen recognition, phagocytosis, antigen presentation, and synapse remodeling (reviewed in Boche et al., 2013). Non-activated microglia termed “quiescent” or “resting” microglia are constantly surveilling the surrounding environment in non-pathological conditions (Nimmerjahn et al., 2005; Marshall et al., 2013). In response to changes in the environment, microglial cells can be activated by changing their morphology and function (Marshall et al., 2013). Their activators include a range of different molecules, such as the P2X7purinergic receptor (P2X7R), and endogenous constituents that are normally released from injured cells, including adenosine 5’-triphosphate (ATP), S100 molecules, histones and heat shock protein (HSP), which are known as damage-associated molecular patterns (DAMPs) (Lu et al., 2014; Wiessinga et al., 2014). Specifically, P2X7R acts as a “sensor of danger” by responding to the so-called “danger signal” ATP, which is released from injured cells and activates microglia (Weisman et al., 2012; Gubert et al., 2013). The same goes for other DAMPs with their specific receptors.

Microglial activation can be divided into two distinct types: a classical M1 and an alternative M2 activation. In the M1 activation, microglial cells may become hyper-ramified or ameboid/phagocytic (Boche et al., 2013), and may synthesize proinflammatory molecules (interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6, among others), superoxide radicals, glutamate (Barger et al., 2007; Takaki et al., 2012), nitric oxide (NO) and ultimately clear infections and repair tissues. Alternatively, M2 activation, which can be triggered by cytokines such as IL-4, IL-13 or IL-25 (Boche et al., 2013; Maiorino et al., 2013), has been associated with a release of anti-inflammatory cytokines such as IL-10, insulin-growth factor-1 (IGF-1), transforming growth factor-β (TGF-β), and neurotrophic factors (Ekdahl, 2012; Boche et al., 2013; Hu et al., 2015), which facilitate healing and limit neuronal injury (Najar et al., 2013). The nature and the magnitude of the injury, along with several other factors, can influence the development of these distinct microglial phenotypes (Marshall et al., 2013). In addition to this dichotomous phenotype classification, a graded level of microglia activation has also been proposed, in which cells can go from a resting stage, to an alert, homing, phagocytic stage and finally to bystander activation, which can be differentiated by morphological features and the levels of cytokines and growth factors secreted (Raivich et al., 1999). Most importantly, identifying activated microglia in a pathological condition, although being a marker of inflammation, does not allow for an understanding of the inflammatory process. Thus, only by determining the phenotype of microglia can one identify its role in cytotoxicity and/or neuroprotection (Colton and Wilcock, 2010; Graeber et al., 2011; Marshall et al., 2013).

Several studies in the past year speculated that alterations in the number and/or morphology of microglial cells are involved in cognitive and behavioral changes observed in psychiatry disorders (Di Benedetto and Rupprecht, 2013; Müller et al., 2015; Nakagawa and Chiba, 2014; Watkins et al., 2014; Zeidan-Chulia et al., 2014; Najar and Pearlman, 2015). However, although activation of microglia is a typical hallmark of brain pathology, the extent to which it has beneficial or detrimental functions in the brain in different psychiatric disorders remains to be elucidated (Dheen et al., 2007). Specifically, given that microglia can be activated in either a cytotoxic or a neuroprotective way, characteristics of the microglial activation assessed in a specific condition need to be taken into account. This review article aims to summarize evidence of inflammation and in major psychiatric disorders, such as major depression, BD, schizophrenia, and autism, including the role it plays in their progression and therapeutics. More specifically, the role of microglial activation and polarization, as well as associated molecular cascades, will also be discussed as a means by which these neuroinflammatory mechanisms take place, when appropriate.

THE ROLE OF MICROGLIA IN STRESS AND DEPRESSION

MDD is considered a critical public health problem, and it is estimated that approximately 350 million individuals are affected worldwide (WHO, 2012). In addition, almost 1 million lives are lost yearly due to suicide, which translates to 3000 suicide deaths every day (WHO, 2012). Until recently, the monoaminergic hypothesis appeared to be the most widely accepted theory for depression. However, a series of new studies have shown that other pathways involved with neuroplasticity or intracellular signaling cascades would be directly or indirectly responsible for the mood dysregulation, as well as to the mechanism of action of antidepressant drugs (Reus et al., 2013a, 2014; Abelaira et al., 2014a,b; Hoyo-Becerra et al., 2014; Ignacio et al., 2014).

Since patients with autoimmune and inflammatory disorders, such as diabetes and fibromyalgia, present with depressive symptoms, it has been proposed that depression may be linked to inflammation (Ceretta et al., 2012; Abelaira et al., 2014a,b; Hoyo-Becerra et al., 2014; Iseme et al., 2014; Mclnnis et al., 2014). In fact, patients with depression have been shown to present an increase in serum levels of proinflammatory cytokines, such as IL-1, IL-6, IL-8, IL-12, interferon-γ (IFN-γ) and TNF-α (Schepers et al., 2005; O’Brien et al., 2007). In addition, elevated plasma levels of IL-1β, IL-1 receptor antagonist, IL-5, IL-6, IL-7, IL-8, IL-10, granulocyte colony-stimulating factor (G-CSF), and IFN-γ have been reported in patients during ongoing depression. Of note, cytokines were reduced to normal levels after 12 weeks of treatment with antidepressants (Dahl et al., 2014). On this same vein, Song et al. (2009) showed an increase in serum IL-1β and a decrease in IL-10 levels (proinflammatory cytokine) in depressed patients. In addition, T-helper 1 (Th1) and T-helper 2
(Th2), which are responsible for pathogens elimination and antibody regulation, respectively, were also found to be altered in untreated depressed patients (Song et al., 2009). Nevertheless, acupuncture and fluoxetine treatments reduced IL-1β levels in responders, whereas acupuncture was also able to restore the balance between Th1 and Th2 systems by attenuating TNF-α concentration and INF-gamma/IL-4 ratio toward the control level (Song et al., 2009). Furthermore, injecting the bacterial endotoxin lipopolysaccharide (LPS) was shown to induce inflammation and to cause subsequent depressive-like behavior in rodents (O’Connor et al., 2009; Ohgi et al., 2013). Even though the underlying mechanisms associated with the onset of inflammation and its relation to the development of depression are not well known, one theory suggests that the inflammatory process in depression emerges from alterations of immune regulators in the CNS. A recent review suggests that psychological stress can activate the immune response in the CNS (Xanthos and Sandkühler, 2014) through enhanced neuronal activity. This would happen through direct interactions between the neurons and the glial cells. Neurons and microglia interact bidirectionally and communicate through fractalkine, a transmembrane chemokine that is expressed by neurons and acts through a receptor (CX3CR1) that is exclusively present on microglia (Xanthos and Sandkühler, 2014; Cardona et al., 2006).

Central immune response is modulated by microglia and astrocytes. Microglia exerts an inflammatory role against danger signals from both the central and peripheral nervous system (McNally et al., 2008; Ranshoff and Perry, 2009; Serrats et al., 2010). Interestingly, an increase in microglial activation and macrophage recruitment was found in postmortem dorsal anterior cingulate matter from individuals suffering from MDD (Torres-Platas et al., 2014). Microglial activation was also greater in the ventral prefrontal white matter in individuals who committed suicide (Schnieder et al., 2014). In the same matter, a correlation between suicide and microglial activation in the dorsolateral prefrontal cortex, anterior cingulate cortex, hippocampus and mediodorsal thalamus from schizophrenic and depressive patients (Steiner et al., 2008) was observed. Altogether, these studies suggest that microglial activation may be considered as an important marker in suicide. Of note, Steiner et al. (2008) did not find any evidence of microglial activation in the same brain areas from patients who were suffering from depression, but did find in patients who had committed suicide, suggesting that microglial activation might be a consequence of presuicidal stress.

Stress has been known to contribute to the development of clinical depression, and evidence from preclinical studies has suggested a role of microglia in depression and stress. Moreover, morphological activation of residential microglia was induced by exposures to acute stress (Sugama et al., 2007). Sprague-Dawley rats exposed to restraint stress showed an increase in microglial activation in the prefrontal cortex, while minocycline, a tetracycline antibiotic, which presents with anti-inflammatory and antidepressant properties (Soczynska et al., 2012; Reus et al., 2014b), was able to reduce the impact of stress on neural and microglial activation (Hinwood et al., 2012). These findings support the theory that microglia plays a pivotal role in modulating the impact of stress. In addition, pro-inflammatory profile and intensified microglial activation were associated with the development of stress-induced anhedonia in susceptible mice (Couch et al., 2013). When changes in neural plasticity occur, the brain becomes susceptible to stress by disrupting glial interactions at the level of the synapse. Indeed, chronic stress promotes microglial hyper-ramification and astroglial atrophy in the prefrontal cortex of rodents (Tynan et al., 2013). In addition, mice exposed to acute stress presented morphological activation of microglia in the thalamus, hypothalamus, hippocampus, substantia nigra and central gray (Sugama et al., 2007). Knockout mouse to IL-18 (−/−), which is a pro-inflammatory cytokine, showed reduced stress-induced morphological microglial activation, indicating a role of IL-18 in restraint stress response (Sugama et al., 2007). It is important to note that animal models of stress are used as a tool to investigate neurobiology of depression and also anxiety. In fact, stress has been linked to the development of both depression and anxiety (Phillips et al., 2015). However, animal models of stress do not always have criteria to validate an animal model, such as face, construct and predictive validities (Abelaira et al., 2013). In the face validity, behavioral changes should be similar to symptoms observed in depressive patients; in the construct validity the pathophysiological changes found in patients should be present in animals; and in the predictive validity behavioral changes should be reversed by effective treatment, such as antidepressants (Abelaira et al., 2013). Thus, studies which showed microglial activation in response to stress could be related to an acute response to stress. In addition, these changes could be related not only to the development of depression, but also anxiety. Long-term changes need to be better investigated in animal models that show criteria for validity. Table 1 presents different animal models of stress and depression and their criteria validity. In addition, microglial alterations induced by stress and the animal models of depression are also summarized.

Prenatal restraint stress in mice was able to induce an increase in IL-1β mRNA levels and in the total number of ionized calcium binding adaptor molecule-1 (Iba1)-immunoreactive microglial cells in the hippocampus. Moreover, when prenatally restraint stressed mice received LPS injections, they presented with an increase in mRNA levels such as IL-6, TNF-α and IP10 (Diz-Chaves et al., 2012). In the same study, the authors showed a higher proportion of Iba1-immunoreactive cells in the hippocampus with morphological characteristics of activated microglia compared to non-stressed mice (Diz-Chaves et al., 2012). Furthermore, prenatally restraint stressed mice presented an increase in TNF-α immunoreactivity in the CA1 region, as well as an increase in the number of Iba-1 immunoreactive microglia and GFAP-immunoreactive astrocytes in the dentate gyrus after LPS administration (Diz-Chaves et al., 2013), thus
sugestig that the hippocampus plays an important role in the inflammatory response induced by stress. Accordingly, the hippocampus is a region with a high density of microglial cells, especially in the CA1 region (Imai et al., 1996). It has been suggested that microglial density is involved in site-specific vulnerability of the hippocampus, and the heterogeneous distribution of microglia might have a role in modulating hippocampal neuronal activity (Jinno et al., 2007; Choi and Won, 2011). Of note, the effects of stress on hippocampal microglial activation have been shown in several studies (Diz-Chaves et al., 2012; Walker et al., 2013a,b; Weber et al., 2015), and it is likely that they might not only be relevant in the pathophysiology of MDD, but also in other psychiatric and stress-related disorders, such as post-traumatic stress disorder (PTSD). PTSD differs from prenatal stress by exacerbating activated microglial cells along with dysfunctional cell proliferation in the hippocampus (Acosta et al., 2013). Nevertheless, Wistar rats exposed to cold stress presented morphological microglial activation and an increase of IL-1β in the hippocampus and the hypothalamus. Additionally, IL-1β was predominantly expressed in the astroglia, rather than microglia, suggesting that exposure to the cold stress may be involved in the communication between the neuron, microglia, and astroglia (Sugama et al., 2011).

Maternal sleep deprivation, an animal model of depression, inhibited neurogenesis through inflammatory cytokines (IL-1β, IL-6 and TNF-α) released from activated microglia in young offspring rats; these effects were associated with memory impairment and anhedonic behavior (Zhao et al., 2014). Prenatal stress induced by the forced swimming (FS) test was able to reduce the number of immature microglia and accelerate microglial differentiation in the neonate Wistar rats, and these effects were associated with an increase in plasma corticosterone in the pregnant rat (Gomez-Gonzalez and Escobar, 2010), thus suggesting that the effects on microglial development and differentiation are mediated by microglial corticosterone receptors. Moreover, corticosteroid receptors are expressed in neurons and also in microglial cells (Tanaka et al., 1997), and it is well known that corticosteroids and their receptors play a pivotal role in stress and depression (Garcia et al., 2009; Reus et al., 2012; Ventura-Junca et al., 2014). Additionally, Frank et al. (2014) demonstrated that chronic corticosterone increased gene expression of inflammasome NLRP3, Iba-1, MHCII, and NF-kBIA, and also potentiated the microglial pro-inflammatory response (TNF-α, IL-1β, IL-6 and NLRP3) to LPS in adrenalectomized (ADX) rodents compared to sham. Thus, it is possible that cortisol signaling in microglia may play an important role in the inflammatory process related to depression (Fig. 1); these effects were dependent on the corticosterone dose, though. In fact, corticosterone administration in low dose was able to reverse microglial activation induced by stress and adrenalectomy (Sugama et al., 2012). Interestingly, treatment with RU486, an antagonist of glucocorticoid receptor, or ADX blocked the sensitization of the microglial pro-inflammatory response induced by stress footshock (Frank et al., 2012). Moreover, glucocorticoid receptors appear to regulate microglial properties during the inflammatory process in the brain (Camillo-de Sauvage et al., 2013). (See Table 2).

Studies have shown that glutamate, an important neurotransmitter in the CNS that plays a key role in the pathophysiology of depression, is also involved in microglial neurotoxicity (Piani et al., 1992; Barger and Basile, 2001). Inflammatory cytokines are able to decrease the expression of the glutamate transporter and increase glutamate release from astrocytes (Miller, 2013). Activation of microglia by inflammatory cytokines, in turn, can induce a release of glutamate that contributes to neuronal damage during neuroinflammation (Barger et al., 2007). Furthermore, cytokines also rouse vesicular release of glutamate from astrocytes, thereby activating presynaptic N-methyl-D-aspartate (NMDA) receptors (Santello and Volterra, 2012) and stimulating indoleamine 2,3 dioxygenase (IDO), which is a potent NMDA agonist and stimulator of glutamate release (Miller et al., 2009). Moreover, glutamate accumulation causes an increase of intracellular Ca^{2+}, which in turn may lead to the production of reactive oxygen species (ROS) due to mitochondrial dysfunction and reduction of antioxidant capacity (Schneider et al., 1996; Stanciu et al., 2000). Thus, mitochondrial dysfunction and oxidative stress contribute to glutamate excitotoxicity and consequently increase proinflammatory genes (Fig. 1). Glutamate and their receptors play an important role in the pathophysiology of depression. Indeed, patients with depression presented a significant increase of serum glutamate levels when compared with healthy controls (Kim et al., 1982; Mitani et al., 2006). In addition, several preclinical and clinical studies have demonstrated that NMDA antagonists, such as ketamine, memantine, amantadine and others present antidepressant effects (Berman et al.,

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Criteria</th>
<th>Microglial activation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restraint stress</td>
<td>–</td>
<td>+</td>
<td>Hinwood et al. (2012)</td>
</tr>
<tr>
<td>Acute stress</td>
<td>–</td>
<td>–</td>
<td>Sugama et al. (2007)</td>
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<tr>
<td>PTSD</td>
<td>–</td>
<td>–</td>
<td>Acosta et al. (2013)</td>
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<tr>
<td>Maternal sleep deprivation</td>
<td>–</td>
<td>+</td>
<td>Gomez-Gonzalez and Escobar (2010), Zhao et al. (2014)</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>–</td>
<td>+</td>
<td>Sugama et al. (2012), Burke et al. (2014)</td>
</tr>
<tr>
<td>Stress footshock</td>
<td>–</td>
<td>+</td>
<td>Catanzaro et al. (2014), Frank et al. (2012)</td>
</tr>
<tr>
<td>LPS</td>
<td>–</td>
<td>+</td>
<td>Walker et al. (2013a,b)</td>
</tr>
<tr>
<td>OB</td>
<td>–</td>
<td>+</td>
<td>Burke et al. (2014)</td>
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Moreover, ketamine has been shown to have anti-inflammatory properties by inhibiting TNF-α and IL-6 gene expression in LPS-activated macrophages (Wu et al., 2008), ultimately abrogating LPS-induced depressive-like behavior (Walker et al., 2013a,b).

Additionally, studies from our group revealed that ketamine reverses the increase of proinflammatory cytokines induced by maternal deprivation (Réus et al., 2015a). In conclusion, it is possible that modulators of the glutamatergic system may play an important role in reducing microglial activation.

Several studies have emerged showing the role of inflammatory modulators in response to stress or microglial activation. In fact, A-804598, an antagonist of P2X7R, has been shown to play an important role in the synthesis and conversion of IL-1β by partially...
attenuating the increased levels of IL-1β and CD14 mRNA in the paraventricular nucleus of rats exposed to stress by footshock (Catanzaro et al., 2014). CD14 acts as a co-receptor (along with the Toll-like receptors TLR 4 and MD-2) for the detection of LPS.

The upregulation of inflammatory cytokines, such as IL-1β and TNF-α, may be suggestive of microglial activation; however, in the case of footshock exposure, these effects do not appear to be mediated totally by P2X7R. Nevertheless, Brilliant Blue G (BBG), another potent P2X7R antagonist, revealed anti-inflammatory properties by decreasing TNF-α levels and antidepressant behavior in the tail suspension (TS) and FS tests (Ma et al., 2014) in rodents after LPS injection. FS and TS are classical tests to investigate new antidepressant drugs (Abeilaira et al., 2013).

Minocycline, which is a suppressor of activated microglia, when chronically administrated, reduces the expression of microglial activation maker CD11b and the M1 pro-inflammatory cytokine IL-1β in the prefrontal cortex of control rats which were not subjected to olfactory bulbectomised (OB), an animal model of depression (Burke et al., 2014). Moreover, minocycline increased the expression of the M2 microglial marker MRC2 and of the associated anti-inflammatory cytokines IL-10 and IL-6 in the prefrontal cortex of OB rats, indicating that the effects of minocycline on microglial markers are dependent on the presence or absence of a depressive phenotype (Burke et al., 2014). Additionally, minocycline potentiated the effects of quercetin, a bioflavonoid with antidepressant properties, in reducing the oxidative stress and microglial activation induced by OB in rodents (Rinwa and Kumar, 2013). Also, minocycline administrated into the cerebral ventricle of rats subjected to learned helplessness (LH) (an animal model of depression) showed antidepressant effects and increased dopamine and its metabolites in the amygdala when compared to untreated rats, but did not alter the LH-induced effects in serotonin turnover and in the BDNF levels in the hippocampus (Arakawa et al., 2012). We also previously demonstrated that minocycline protected against oxidative damage in the brain of rats subjected to chronic mild stress (Reus et al., 2015b). Thus, minocycline might be promising as a therapeutic target to treat depression by reducing microglial activation, oxidative stress and inflammation.

Antidepressant drugs used to treat depression also act in microglial regulation. In fact, selective serotonin reuptake inhibitors (SSRIs) potently inhibit microglial TNF-α and NO production induced by LPS administration, and cAMP signaling was involved in regulating this anti-inflammatory response (Tynan et al., 2012). Fluoxetine, an SSRI, also reduced the microglial activation in dopaminergic neurons induced by an animal model of Parkinson (Chung et al., 2011), and also prevented LPS-induced degeneration of nigral dopaminergic neurons by inhibiting microglia-mediated oxidative stress (Chung et al., 2010).

In conclusion, there are consistent data to indicate that immune system dysregulation and microglial activation may be key elements in mood dysregulation. Moreover, the antidepressant effects of classic and new modulators could be mediated, at least in part, by its effects on regulating the immune system. However, how such effects may occur is not yet well understood. Therefore, future studies focusing on the association between immune activation and stress could help in the development of new therapeutic targets for depression.

**THE ROLE OF MICROGLIA IN BD**

BD is a severe mood disorder characterized by recurrent episodes of mania followed by depression. Although the clinical characteristic for the diagnosis of BD is the presence of manic symptoms, depression represents the predominant mood state in patients with BD type I and BD type II. The pathophysiology of BD has been attributed to deficits in monoamine neurotransmitters, such as dopamine. However, the neurobiology of BD, as well as the mechanism of action of mood stabilizers used to treat BD, is not yet fully elucidated. Thus, it is possible that other pathways besides the monoaminergic system could be involved. Recently, mood disorders are increasingly being recognized as inflamed moods (Rosenblat et al., 2014). One theory suggests that in BD the immune system is chronically activated by microglia, which in turn produces cytokines that render the brain to a vulnerable and unstable state, precipitating mood disturbances (Schoeter et al., 2011). In fact, higher levels of IL-1β were associated with dysfunction and increased suicide risk in patients with BD (Monfrim et al., 2014). Changes in sleep pattern were also observed in patients with BD, with an increase of IL-6 in peripheral monocytes (Ritter et al., 2013). Furthermore, Barbosa et al. (2013) demonstrated an increase in proinflammatory cytokines’ levels in BD. In euthymic patients with BD, an increase in blood kynurenine concentrations and in the kynurenine to tryptophan ratio was also observed (Reininghaus et al., 2014). The kynurenic pathway plays an important role in psychiatric diseases; this pathway is an alternate route of tryptophan metabolism that decreases serotonin neurotransmission (Watkins et al., 2014). Moreover, stimulated microglia may promote expression of cytokines, such as IFN-γ, a potent activator of Kynurenine pathway (KP). IFN-γ increases the activity of indoleamine 2,3-dioxygenase (IDO), consequently increasing quinolinic acid (QUIN) (Watkins et al., 2014), which causes excitotoxicity mediated by the NMDA receptor. In the CNS, activated microglia and infiltrating macrophages are considered the main producers of QUIN (Heyes et al., 1996).

Due to the complexity of BD, most studies using animal models focus on the effects that mood stabilizers and antipsychotics have in the microglial cells. Valproate (VPA), a histone deacetylase inhibitor (HDACi), seems to play an important role in managing activated microglial cells. Cells pretreated with VPA showed decreased levels of proinflammatory factors, which were concentration- and time-dependent (Peng et al., 2005). The anti-inflammatory effects of VPA appear to be based on its ability to induce apoptosis in activated microglia. VPA is capable of inducing cell apoptosis in brain.
microglia as well as in the microglial cell line BV-2. Moreover, the apoptosis is possibly mediated by p38 mitogen-activated protein kinase (MAPK) and mitochondrial apoptosis pathway (Xie et al., 2010). Additionally, pre-treatment with HDACi sodium butyrate (SB) and trichostatin A (TA) reduced LPS-induced dopaminergic (DA) neurotoxicity in mesencephalic neuron-glia cultures (Chen et al., 2006, 2007). It is proposed that this effect in LPS-induced activated microglia may cause an increased deacetylation of astroglial histone proteins. Therefore, HDACi would be able to restore the down-regulation of antioxidant capacity induced by inflammation in astrocytes, as well as reduce cell death following oxidative stress (Correa et al., 2011). Lithium, a mood stabilizer, was also able to significantly inhibit LPS-induced microglial activation and pro-inflammatory cytokine production in vitro. Lithium pretreatment was able to suppress LPS-induced toll-like receptor 4 (TLR4) expressions via the PI3K/Akt/FoxO1 pathway (Dong et al., 2014). Altogether, these results point to an interesting relationship between the mechanism of action of these classic mood stabilizers (Lithium and VPA) and microglial cells.

Despite the pharmacological results, there is lack of research characterizing the microglial population (number and level of activation) in BD patients, even though there is an increase in the number of studies suggesting its involvement in this pathology (Beumer et al., 2012; Rege and Hodgkinson, 2013; Stertz et al., 2013). Most of the current studies focus mainly on other types of glial cells, such as astrocytes and oligodendrocytes (Gigante et al., 2011; Savitz et al., 2014). A recent study showed a significant increase in [11C]-R-PK11195 binding potential, which is indicative of microglia activation and neuroinflammation. This increase was found in the right hippocampus of patients with BD type I compared to healthy controls (Haarman et al., 2014). Still, other independent studies with this marker are necessary to corroborate this finding.

Regarding postmortem studies, increased markers of excitotoxicity and neuroinflammation in the frontal cortex of BD patients were demonstrated by Rao and cols. The authors showed significantly higher protein and mRNA levels of IL-1β, IL-1 receptor (IL-1R), myeloid differentiation factor 88, nuclear factor-kappa B subunits, astroglial and microglial markers (glial fibrillary acidic protein, inducible nitric oxide synthase (iNOS), c-fos, and CD11b) in these patients (Rao et al., 2010). Contrary to these findings, another study focusing on the prefrontal white matter found an increased density in oligodendrocyte, but not in the density of lba-1-stained microglia in BD samples. Regarding the qualitative assessment of microglial morphology, the study found numerous activated microglial cells in schizophrenia samples, but not in controls or BD samples (Hercher et al., 2014). Furthermore, an evaluation of microglial cells in the amygdala of BD patients using stereological methods also failed to demonstrate differences in cell numbers compared to healthy controls (Hamidi et al., 2004). In conclusion, despite a lot of speculation, in order to finally prove that microglial cells are involved in the pathophysiology of the disorder and/or its progression, new tools to target the microglial activation in the human brain needs to be developed.

MICROGLIAL HYPOTHESIS OF SCHIZOPHRENIA

Schizophrenia is a chronic and debilitating disorder that affects 0.5–1% of the world population (Tandon et al., 2008). Patients with this disorder present positive and negative symptoms. Positive symptoms are characterized by extra feelings or behaviors, such as hallucinations and delusions. On the other hand, negative symptoms are associated with lack of behaviors, for example, apathy and loss of interest in everyday activities. Evidence suggests that the dopamine dysfunction hypothesis, which involves hyperstimulation of dopaminergic D2 receptors in certain parts of the brain, may lead to positive symptoms. The glutamatergic hypofunction hypothesis of schizophrenia could be responsible for the negative symptoms (Meyer, 2013). Additionally, neuroinflammation has been linked to schizophrenia, as well (Monji et al., 2009). One theory suggests that maternal immune activation during pregnancy is a risk factor for the progeny to develop schizophrenia in adulthood (Brown, 2011). The findings from preclinical studies using models of prenatal infection and maternal immune activation through polyinosinic-polyricydlic (Poly I:C) or LPS can have a negative impact on offspring brain development (Missault et al., 2014; Reisinger et al., 2015; Wischhof et al., 2015). Van den Eynde et al. (2014) demonstrated that offspring rats born to Poly I:C had an increase in microglia accompanied by schizophrenic-like behavior. Influenza exposure during the first gestational trimester significantly increased the risk of schizophrenia in adulthood (Brown et al., 2004). Other studies showed an association between Toxoplasma gondii and early-onset schizophrenia (Mortensen et al., 2007), and maternal genital/reproductive infections during periconception increased the risk of schizophrenia in offspring (Babulas et al., 2006). On this vein, several models have attempted to explain how prenatal infection can increase the risk of schizophrenia. A theory suggests that the host immune response through cytokines could mediate the effects of infection (Girgis et al., 2014). During infection the innate immune cells are also activated by endogenous constituents that are normally released from injured cells, including ATP, S100 molecules, histones and HSPs, which are known as DAMPs (Lu et al., 2014; Wiersinga et al., 2014). Thus, microglia can be stimulated by numerous components including DAMPs or pro-inflammatory mediators to produce cytokines, chemokine, and induce oxidative stress (Fig. 2). This prolonged and excessive microglial response may lead to deleterious effects on neuronal plasticity and apoptosis, leading to behavioral and cognitive deficits through exogenous as well as endogenous components (Barichello et al., 2013; Hu et al., 2014).

Microglial activation and an increase in microglial cells in the brain of schizophrenic patients have been reported in post-mortem studies (Bayer et al., 1999; Radewicz et al., 2000). An increase in microglial cells was also
demonstrated in schizophrenic patients who had committed suicide (Steiner et al., 2008). A positron emission tomography (PET) study showed microglial activation in recent-onset schizophrenics within the first 5 years of disease (van Berckel et al., 2008). Moreover, microglial activation through Iba-1 and iNOS in the hippocampus of adult rats was observed in an animal model of schizophrenia induced during the neonatal period (Fig. 2). This microglial activation was accompanied by prepulse inhibition (PPI) and working memory impairment that were reversed by antipsychotic clozapine treatment (Ribeiro et al., 2013). However, in the prefrontal white matter from schizophrenic patients, Iba-1 expressing microglial cells were found only in three out of 20 schizophrenia samples compared to controls (Hercher et al., 2014).

Schizophrenia is known to be associated with alterations in the immune system, such as increased cytokine levels. In a meta-analysis, IL-1β, IL-6, and TGF-β (which also has neuroprotective and anti-inflammatory effects in the CNS) were augmented during an acute relapse in patients and in the first episode of psychosis. These alterations in cytokines were normalized with antipsychotic treatment (Fig. 2) (Miller et al., 2011). Another meta-analysis revealed a link between polymorphism in the IL-1β gene and abnormal white and gray matter volume in schizophrenia (Najjar and Pearlman, 2015).

Schizophrenic patients have also shown DAMPs in the serum or in the cerebrospinal fluid (CSF). Micromolar concentrations of S100B protein (a protein involved with cell cycle progression and differentiation) can induce apoptosis in neurons and astrocytes in response to the activation of the advanced glycation end-products receptor (RAGE) (Steiner et al., 2009). S100B protein has been shown to be augmented in the serum and in the CSF of untreated schizophrenic patients (Schmitt et al., 2005).

Heat shock 70-kDa proteins (HSP70s) are molecular chaperones and also microglial activators, which regulate biological processes and are associated with the pathophysiology of schizophrenia. Antibodies against HSP70 have been found in the serum of schizophrenic patients, which is suggestive of inflammation (Kim et al., 2001, 2008). Extracellular HSP70 mediates the innate immune responses of brain through toll-like receptors (TLRs). TLRs activate the nuclear transcription factor kappa B (NF-κB), which plays a key role in the expression of genes responsible for the development of cell and inflammation. These transcription factors also are capable of activating the promoter region of many pro-inflammatory genes, including genes expressed by the M1 microglial phenotype (Tato and Hunter, 2002; Saijo and Glass, 2011).

In conclusion, inhibition of pro-inflammatory cytokines or enhancement of anti-inflammatory mediators in schizophrenic patients may be a beneficial strategy to prevent the devastating consequences of this illness with respect to neuronal damage and function. We propose that inhibition of the activity of DAMPs and/or administration of specific anti-inflammatory agents may lead to amelioration of symptoms of this debilitating illness.


**MICROGLIAL ACTIVATION IN AUTISM**

The autism spectrum disorders (ASD) are neurodevelopmental disorders, which are characterized by language and intelligence deficits, as well as impairment in social interactions (Abrahams and Geschwind, 2008; Theoharides et al., 2013). Recent studies have demonstrated a relationship between autism and inflammation dysregulation/alteration (Young et al., 2011; Theoharides et al., 2013). Microglial activation has also been reported in patients with ASD. For instance, Tetreault et al. (2012) reported an increase of microglial cells in cortical areas (fronto-insular (FI) and visual cortex (VC)) from individuals diagnosed with autism. Moreover, in a postmortem study microglia was found to be augmented in individuals with autism when compared with healthy controls (Morgan et al., 2012), and a PET revealed that young adults with ASD had increased markers of microglial activation in a wide range of brain areas, including the cerebellum, brainstem, frontal cortex, anterior cingulate cortex, corpus callosum, temporal cortex, and parietal cortex (Suzuki et al., 2013). Dendritic cells, which are important in modulating immune responses, were found to be increased in the amygdala of individuals with ASD in a magnetic resonance imaging (MRI) study (Breece et al., 2013).

A study with mice lacking the chemokine receptor CX3CR1 demonstrated deficits in microglia, which were associated with impairments in neural plasticity and social interaction, which are linked with autism (Zhan et al., 2014). Moreover, prenatal LPS exposure induced autistic-like behavior and dopaminergic hypoactivity; however, the expression of glial cell markers in the striatum was not altered (Kirsten et al., 2012). Conversely, Le Belle et al. (2014) revealed that pregnant mice exposed to LPS had offsprings with increased forebrain microglia and autistic behavior. In addition, LPS exacerbated glial activation in the hippocampus and the cerebellum, as well as behavioral changes induced by the VPA-induced animal model of autism in the gestational period (Lucchina and Depino, 2014). Thus, it is possible that prenatal inflammation might be involved, at least in part, to microglial activation in ASD (Fig. 3).

Rett syndrome, which is a X-linked ASD and is a devastating neurodevelopmental disorder, was also shown to be related to microglial alteration. In fact, Derecki et al. (2012) demonstrated brain microglial activation in a murine model of Rett syndrome. This effect was inhibited by annexin V, which blocks phosphatydilserine residues on apoptotic targets. In addition, damages in dendrites and synapses and microglial activation due to high levels of glutamate were found in an animal model of Rett syndrome (Maezawa and Jin, 2010). The same authors demonstrated that inhibition of microglial glutamate synthase, release or antagonism was able to block its neurotoxicity activity in the hippocampus (Maezawa and Jin, 2010). Glutamate dysfunction has been reported also in ASD. A study showed that autistic patients exhibited higher glutamate concentration; however, higher GABA and lower GABA/glutamate have been found in the patients (El-Ansary and Al-Ayadhi, 2014). Additionally, a decrease in TNF-α and IL-6 and an increase in IFN-γ and IFI16 (a neuroinflammatory marker) were demonstrated (El-Ansary and Al-Ayadhi, 2014).

Altogether, these results suggest that an imbalance in GABA and glutamate neurotransmission might be related to neuroinflammation in ASD. Thus, glutamatergic modulators could be promising to treat ASD spectrum to ultimately reduce glutamatergic excitotoxicity and consequently microglial activation (Fig. 3).

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**Fig. 3.** The role of neuron-glia, glutamatergic signaling and immune system interactions in the pathophysiology of ASD and Rett syndrome. Prenatal infection with LPS may lead to microglial activation. Microglia may be also activated by higher levels of Glu, as well as increase in Glu levels. Elevated glutamate levels from presynaptic neuron and microglia may increase Glu levels in the synaptic cleft and consequently intraneuronal Ca\(^{2+}\) levels via NMDA receptors, which may be involved with neuroinflammatory protein activation, thus leading to a vicious cycle with elevation of proinflammatory cytokine levels. These toxic effects could be involved with autism and Rett syndrome disease and neuroprogression. On the other hand, decrease in Glu levels by glutamatergic modulators act to reduce microglial activation and pro-inflammatory cytokine production. Glu = glutamate; LPS = lipopolysaccharide; NMDA = N-methyl-D-aspartate.
Recent studies show that there is a potential role of microglial activation in autism and Rett syndrome, and the pathways linked to microglial activation may be involved with the neurodevelopment of both autism and Rett syndrome. Moreover, preclinical studies in this area may help to comprehend the mechanisms by which microglial activation may be involved with the autistic spectrum.

CONCLUSION

Microglial activation and neuroinflammation are evident in psychiatric conditions and have been reported by preclinical and clinical studies. However, the pathological mechanisms involved in the microglial dysfunction are still not fully elucidated. Of note, it remains unclear whether microglia activation can lead to the onset of psychiatric disorders and consequently to a neuroinflammatory process. More specifically, even though microglial activation can present two opposite phenotypes, the majority of the studies do not take these phenotypes into account when assessing microglial activation in different disorders. In other words, we can only infer whether a particular disorder is associated with the M1 or M2 phenotype based on the reported inflammatory markers that the patients present. In addition, many studies show peripheral inflammation in psychiatric disorders; however, increased inflammatory markers in the periphery are not synonyms of microglial activation in the CNS. Thus, future studies are needed to better characterize peripheral inflammatory markers involved with microglial activation and to describe the role of microglia and their key regulators in the symptomatic manifestations of psychiatric disorders. Future studies may also help in the development of new therapeutic targets, both for prevention and for treatment.

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