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The impact of stress on immune systems and its relevance to mental illness

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Highlights

- Chronic stress mobilizes leukocytes via sympathetic nerves and glucocorticoids.
- Leukocytes then secrete cytokines and infiltrate the brain for emotional changes.
- Chronic stress activates microglia via innate immune receptors TLR2/4.
- Microglia then cause emotional changes via multiple neuro-immune pathways.
- Similar immune system abnormalities in the brain and periphery occur in depression.

Keywords: Depression, Inflammation, Innate immunity, Leukocytes, Microglia, Stress

38 pages including 3 figures and 1 table

Abstract

Stress due to adverse and demanding conditions alters immune functions. How innate and adaptive immune systems respond to stress and affect neural processes remains unclear. Rodent studies have demonstrated crucial roles of stress-induced immune responses for depressive- and anxiety-like behaviors. In the periphery, stress evokes the mobilization of neutrophils and monocytes to the circulation via sympathetic nerves and glucocorticoids. These myeloid cells are thought to promote depressive- and anxiety-like behaviors by infiltrating the brain's perivascular space, releasing cytokines, and affecting vascular endothelial functions. In the brain, stress activates microglia via innate immune receptors TLR2/4. The activated microglia in the medial prefrontal cortex secrete cytokines and alter neuronal morphology and activity in their vicinity. In subcortical brain areas, prostaglandin (PG) E_2 released from the activated microglia attenuates the dopaminergic projection to the medial prefrontal cortex via PGE receptor EP1. These multiple actions of microglia promote depressive-like behavior in concert. These rodent findings may be translatable to depression that clinical studies have associated with brain and peripheral inflammations. Understanding causal relationships between immune and neural alterations under stress might be exploitable to develop inflammation-targeting therapeutics for mental illness.

1. Introduction

Stress is a strain of mental and physical functions caused by adverse and demanding conditions inside and outside the body. Stress evokes adaptive biological responses to cope with it for wellbeing and survival. However, if it becomes excessive or prolonged, stress may cause depression, elevated anxiety, and cognitive dysfunctions, increasing the risk of mental and physical illnesses (Yaribeygi et al., 2017; McEwen et al., 2017). Many recent studies on stress have focused on its effects on brain functions, leaving its impact on other systems less explored. Nonetheless, studies have shown that stress affects immune systems since Hans Selye proposed the concept of stress in the 1950s (Szabo et al., 2012). He discovered that stress causes the atrophy of the thymus, a crucial organ for adaptive cellular immunity. He and later researchers also found that stress activates the hypothalamic-pituitary-adrenal (HPA) axis as represented by blood glucocorticoid increase and adrenal hypertrophy. Since glucocorticoids suppress various responses of immune cells, stress was considered immunosuppressive. Clinical studies corroborated this notion by suggesting that chronic stress suppresses adaptive cellular and humoral immunity, the latter of which being less affected under acute stress (Segerstrom et al., 2004). Rodent studies also confirmed the suppressive effect of stress on adaptive immunity to infection (Glaser et al., 2005). However, stress may augment innate immune responses under certain if not all conditions. Several studies in mice showed that repeated social defeat stress mobilizes neutrophils and monocytes from the bone marrow to the circulation (Engar et al., 2004; Powell et al. 2013; Xu et al., 2020; Ishikawa et al., 2021). Stress reportedly enhances the bactericidal activity of splenic macrophages and innate immune response to viral infection (Bailey et al., 2007), although it could also counteract this effect via sympathetic nerves (Andersson & Tracey, 2012; Abe et al., 2017). Besides,

stress activates microglia, increasing the expression of proinflammatory mediators in the brain and induces brain infiltration of monocytes through the blood-brain barrier (Weber et al., 2017). Depressive patients also show abnormalities in innate immune systems in the periphery and brain, as exemplified by increased neutrophils, monocytes, and inflammatory mediators in the blood (Hasselmann et al., 2018; Lynall et al., 2020; Syed et al., 2018) and neuroinflammation in specific brain areas visualized by PET imaging (Setiawan et al., 2015; Setiawan et al., 2018), respectively.

This review summarizes the impact of stress on immune systems in the periphery and brain and their behavioral relevance. We also discuss translating these stress responses in rodents to stress pathology in mental illness, such as depression.

2. Stress-induced immune responses in the periphery and their mechanisms

2.1. Stress-induced leukocyte mobilization from the bone marrow

Studies in rodents, mostly with repeated social defeat stress, have shown that stress alters leukocyte counts, distribution, and properties (Table 1, Figure 1) (Davis et al., 2008; Ince et al., 2019). Generally, chronic stress decreases lymphocytes, such as T cells and B cells, whereas it increases myeloid cells, such as neutrophils and monocytes, in the blood and bone marrow (Engar et al., 2004; Powell et al., 2013; Xu et al., 2020; Ishikawa et al., 2020). These changes could be due to altered differentiation and proliferation of leukocytes in the bone marrow and their egress to the circulation. However, not all leukocyte subsets behave similarly after the cessation of stress. The neutrophil increase and B cell decrease sustain for several days after the last stress, whereas other leukocyte changes disappear (Ishikawa et al., 2020). Acute stress could induce similar changes in leukocytes as chronic stress, as lymphocyte decrease and myeloid cell increase were

noticeable immediately after single 2-hour exposure to social defeat stress. However, the effects of acute stress may be less sustained. Only neutrophil increase, but not monocyte increase, was observed 12 hours after single 2-hour exposure to social defeat stress (Wohleb et al., 2013). These findings indicate that chronic stress induces longer-lasting leukocyte changes than acute stress and that neutrophil increase sustains longer than the changes in other leukocyte subsets.

2.2. Roles of autonomic and neuroendocrine systems

Stress-induced mobilization of these cells from the bone marrow is mainly mediated by autonomic and neuroendocrine systems (Figure 1) (Glaser et al., 2005). The activation of the sympathetic nervous system upon stress induces the release of noradrenaline and adrenaline to the blood and tissues. These neurotransmitters activate adrenergic receptors on leukocytes and their surrounding cells (e.g., mesenchymal stem cells in the bone marrow), leading to stress-induced leukocyte mobilization (Heidt et al., 2014). Sympathetic activation could be sufficient for these effects, as systemic treatment with isoprenaline, a non-selective β-adrenergic agonist, decreases lymphocytes and increases monocytes and granulocytes in the bone marrow (McKim et al., 2018). Pharmacological inhibition of β2 and β3 adrenergic receptors suppresses stress-induced mobilization of myeloid cells, suggesting their crucial role. These adrenergic receptors could promote the differentiation and proliferation of myeloid cells (Vasamsetti et al., 2018). They could also reduce C-X-C motif ligand (CXCL) 12 expression on osteoblasts and mesenchymal stem cells that retain myeloid cells in the bone marrow via C-X-C motif chemokine receptor (CXCR) 4 (Katayama et al., 2003; Heidt et al., 2014).

Besides, stress activates the HPA axis and increases the level of glucocorticoids in the blood (Figure 1). Glucocorticoids directly act on myeloid and lymphoid cells to suppress

inflammatory responses (Cain & Cidlowski, 2017). They also promote apoptosis of T cells, macrophages, and dendritic cells, leading to inhibition of immune responses (Amsterdam et al., 2002). However, after chronic stress, monocytes and macrophages develop glucocorticoid resistance, or reduced sensitivity to glucocorticoids, in mice and humans, thereby disinhibiting inflammatory responses (Weber et al., 2017). Alongside, glucocorticoids promote innate immune responses upon stress by suppressing the CXCL12-CXCR4 pathway that retains myeloid cells in the bone marrow and augmenting cytokine release from these cells, since metyrapone, a glucocorticoid synthesis inhibitor, prevented all these responses (Niraula et al., 2018).

2.3. Roles of cytokines and chemokines

Stress affects the expression of various cytokines and chemokines in the bone marrow and circulation, likely contributing to stress-induced immune responses (Figure 1). Distinct yet overlapping cytokines regulate the proliferation and mobilization of neutrophils and monocytes. G-CSF generally promotes neutrophil proliferation and mobilization, though not exclusively. G-CSF is thought to activate the sympathetic nervous system locally in the bone marrow, leading to neutrophil mobilization (Asada et al., 2013). CXCL1/2 and their receptor CXCR2 also stimulate neutrophil mobilization (Hong, 2017). M-CSF and GM-CSF augment the proliferation and mobilization of monocytes, and C-C motif chemokine 2 (CCL2) and its receptor C-C chemokine receptor type 2 (CCR2) stimulate monocyte mobilization (Gordon & Taylor, 2005). Stress increases the levels of G-CSF in the blood (Xu et al., 2020). As IL-17 promotes neutrophil mobilization via G-CSF secretion, restraint stress increases aged neutrophils via IL-17A, presumably derived from intestinal T helper 17 (Th17) cells (Xu et al., 2020). Since this neutrophil mobilization depends on segmented filamentous bacteria in the gut, IL-17A

could link gut microbiota and innate immune response to stress (Figure 1). Indeed, stress affects gut microbiota that is associated with and influences stress susceptibility (Yang et al., 2017; Foster et al., 2017). Although its mechanism remains elusive, gut microbiota have been shown to be regulated by multiple routes, such as vagus nerves, glucocorticoids, leukocytes, and secreted cytokines (Rea et al., 2020). However, the effects of stress on circulating IL-17 levels vary among studies, and whether IL-17A mediates neutrophil mobilization under stress remains to be established. Besides, stress increases the CXCL2 and CCL2 levels in the blood and brain (Sawicki et al., 2015; Elkhatib et al., 2020). This CCL2 increase is not essential for stress-induced monocyte mobilization to the circulation since CCR2 deletion did not affect it (Wohleb et al., 2013). Instead, the CCL2 increase in the brain is crucial for stress-induced monocyte infiltration to the brain.

2.4. The effects of stress on immune systems via multiple organs

Stress can affect the spleen and lymph nodes via the projection of the sympathetic nervous system, at least in part (Figure 1). Acute stress reportedly induces noradrenaline release from sympathetic nerve endings in the spleen (Abe et al., 2017), which acts on choline acetyltransferase-positive ($ChAT^+$) T cells via β2 adrenoceptors (Andersson & Tracey, 2012). These spleen T cells then release acetylcholine and inhibit the production of proinflammatory cytokines by macrophages. This anti-inflammatory pathway is beneficial in various inflammatory disease models, including ischemic acute kidney injury and can be activated by vagus nerves connected to splenic sympathetic nerves (Andersson & Tracey, 2012). Chronic stress, such as repeated electronic foot shock or restraint stress, increases $CD4^+$ T cells with reduced glycolysis and oxidative phosphorylation as well as mitochondrial fission in the spleen and lymph nodes (Fan et al., 2019). This metabolic remodeling appears to contribute to stress-induced elevated

anxiety (see later for details). Chronic stress may also increase splenic plasma cells and enhance antibody-dependent immune responses by activating stress-related brain regions, such as the central nucleus of the amygdala and paraventricular nucleus of hypothalamus, and splenic nerves (Zhang et al., 2020). This enhancement of humoral immunity occurs only when the stress is weak and does not evoke glucocorticoid release, such that it does not occur with restraint stress accompanied by glucocorticoid release. Thus, humoral immunity is differentially regulated depending on stress conditions. Stress can also affect non-immune peripheral organs, as acute stress reportedly increases IL-6 release from brown adipocytes through direct sympathetic actions (Figure 1) (Qing et al., 2020).

Collectively, the interactions among multiple organs, including the bone marrow, spleen, gut, and brown adipose tissue, shape stress-induced innate and adaptive immune responses in a manner depending on stress conditions (Figure 1).

3. The effects of stress-induced immune responses in the periphery on neural functions

3.1. Roles of bone marrow-derived myeloid cells

Stress-induced immune responses described above affect neural functions and contribute to stress-induced behavioral changes (Figure 2). There are multiple barriers between the brain and periphery, and leukocytes rarely exist in the brain parenchyma in a healthy condition. Nonetheless, myeloid cells, such as monocytes, are thought to infiltrate the perivascular spaces of the brain, especially under chronic stress (Wohleb et al., 2013; Menard et al., 2017). Circulating monocytes adhere to vascular endothelial cells in the brain and enter the perivascular space (Sawicki et al., 2015; Zheng et al., 2016). Alongside, an elegant study with skull transplantation has demonstrated the direct route of myeloid

cells from skull bone marrow to dura mater, skipping the entry to the circulation (Cugurra et al., 2021). Dural myeloid cells would yet have to penetrate another type of barrier, arachnoid and pia maters underneath, to reach the brain parenchyma. Repeated social defeat stress increases perivascular monocytes in a manner dependent on CCR2 (Wohleb et al., 2013). CCL2, a CCR2 ligand, is reported to be increased in microglia after repeated social defeat stress, suggesting that microglia-derived CCL2 recruits perivascular monocytes via CCR2 (Figure 2). Mice lacking CCR2 attenuate elevated anxiety induced by repeated social defeat stress and concomitant brain infiltration of monocytes, suggesting the anxiogenic role of blood-borne monocytes. Repeated social defeat stress also mobilizes neutrophils into the circulation, and this neutrophil increase sustains longer after the cessation of stress than that of monocytes (Ishikawa et al., 2020). Notably, the neutrophil increase is more pronounced in BALB/c mice than in C57BL/6N mice. Since BALB/c mice are more susceptible to stress than C57BL/6N mice, stress-induced neutrophil increase could mediate genetic stress susceptibility.

3.2. Roles of circulating cytokines

Stress-mobilized myeloid cells appear to affect neural functions via the secretion of cytokines (Figure 2) (London et al., 2013). IL-6 is one of these cytokines since bone marrow transplantation from IL-6 knockout mice reduces depressive-like behavior (Hodes et al., 2014). Notably, single 10-min exposure to social defeat stress increases IL-6 in the blood before depressive-like behavior develops. This IL-6 increase is larger in mice that will eventually develop depressive-like behavior after the repeated stress (i.e., susceptible mice) than in those that will not (i.e., resilient mice). Thus, pre-existing variability of stress-induced IL-6 release from leukocytes could contribute to stress susceptibility. Circulating IL-6 appears to leak into the brain parenchyma through the

blood-brain barrier (BBB) since repeated social defeat stress disrupts the BBB with reduced claudin-5 responsible for tight junctions in endothelial cells of the brain vasculature (Menard et al., 2017). IL-1β and its receptor, IL-1 receptor type I (IL-1RI), are also involved in stress-induced depressive- and anxiety-like behaviors. IL-1β derived from leukocytes appears crucial for elevated anxiety since bone marrow transplantation from mice lacking caspase-1, an enzyme responsible for IL-1β maturation, abolished this behavioral change (McKim et al., 2018). Leukocyte-derived IL-1β could act on IL-1RI on the brain vasculature to cause its anxiogenic effects, as shown by endothelial cell-specific knockdown of IL-1RI that impaired stress-induced elevated anxiety. These findings illustrate that leukocyte-derived cytokines affect neural functions via multiple routes, directly or indirectly (Figure 2).

3.3. Roles of T cells and other immune cells

As described above, stress also increases $CD4^+$ T cells with a metabolic remodeling in the spleen and lymph nodes. $CD4^+$ T cells are crucial for chronic stress-induced anxiety-like behavior, as demonstrated by the loss of this behavior with antibody-mediated depletion of $CD4^+$ T cells as well as genetic Rag1 deletion (Fan et al., 2019). Adoptive transfer revealed that naïve $CD4^+$ T cells from stressed mice are sufficient to restore the anxiety-like behavior in Rag1 knockout mice, suggesting unconventional functions of adaptive immune cells (Figure 2). Mitochondrial fission in $CD4^+$ T cells under chronic stress could underlie stress-induced anxiety-like behavior since genetic induction of mitochondrial fission in these cells causes anxiety-like behavior even without stress. The anxiogenic effect of $CD4^+$ T cells with mitochondrial fission depends on purine nucleoside phosphorylase 2 in the xanthine oxidase pathway, implicating abnormal purine metabolism in this behavioral effect.

Immune cells, including macrophages and resident lymphocytes, exist around the barriers between the brain and periphery and could be involved in neuroinflammation (Kierdorf et al., 2019). These macrophages are called CNS-associated macrophages (CAMs), classified into perivascular, meningeal, and choroid plexus macrophages (Hove et al., 2019). It was recently reported that meningeal γδ T cells regulate anxiety-like behavior via IL-17A signaling in prefrontal neurons (Lima et al., 2020). It remains unexplored whether stress affects meningeal and choroid plexus macrophages and resident lymphocytes and whether they are involved in stress-induced neural dysfunctions.

4. Stress-induced neuroinflammation

4.1. Roles of microglia and their inflammatory substances

Stress induces not only immune responses in the periphery but also inflammatory responses in the brain (Figure 3). Stress-induced neuroinflammation and its significance have been explored since the discoveries that stress induces the expression of proinflammatory cytokines, such as IL-1β, in the brain (Minami et al. 1991; Nguyen et al. 1998). IL-1 signaling is crucial for chronic stress-induced depressive- and anxiety-like behaviors since IL-1RI knockout mice failed to show these behaviors (Koo et al., 2008). Prostaglandin (PG) E_2, a lipid mediator derived from arachidonate, is also involved, as shown by mice lacking PGE receptor EP1 that failed to show these behavioral changes (Tanaka et al., 2012).

These inflammatory substances are mainly derived from microglia, resident macrophages in the brain. Microglia are highly motile even without brain damages and play various physiological functions (Wake et al., 2013). Consistent with microglial involvement, chronic stress, including chronic unpredictable stress and repeated social

defeat stress, activates microglia in specific brain areas (Figure 3). This microglial activation is at least in part via innate immune receptors called Toll-like receptors (TLRs). TLRs recognize not only pathogen-associated molecular patterns (PAMPs) but danger-associated molecular patterns (DAMPs), endogenous ligands released from the cell upon cellular damage or inflammatory stimuli. TLR2 and TLR4 (TLR2/4) are crucial for stress-induced depressive- and anxiety-like behaviors and dendritic shrinkage and the attenuated response of prefrontal neurons since genetic TLR2/4 deletion abolishes these changes (Nie et al., 2018). As TLR2/4 are highly expressed in microglia, they are also necessary for concomitant microglial activation. Notably, selective knockdown of TLR2/4 in prefrontal microglia inhibits depressive-like behavior, demonstrating the behavioral significance of prefrontal microglial activation. The activated microglia promote depressive-like behavior via proinflammatory cytokines, IL-1α and TNF-α (Figure 3). How these cytokines affect neuronal structures and functions warrants future investigations.

TLR2/4 are also crucial for stress-induced prostaglandin (PG) E_2 increase in the brain. PGE_2 is a lipid mediator derived from arachidonate involved in inflammation and immune responses. Multiple biochemical pathways can synthesize PGE_2 from arachidonate-containing membrane phospholipids or endocannabinoids, respectively (Park et al., 2006). In the brain, monoacylglycerol lipase (MAGL) metabolizes 2-arachidonoylglycerol to free arachidonate, which is further converted to PGE_2 by cyclooxygenase (COX) and PGE synthase (Nomura et al., 2011). Microglia that express COX-1, one of the two COX isoforms, could be the source of PGE_2 under stress since pharmacological inhibition and genetic deletion of COX-1 abolishes stress-induced brain PGE_2 increase and depressive-like behavior (Tanaka et al., 2012; Nie et al., 2019). Pharmacological MAGL inhibition

also abolishes both of these (Nie et al., 2019). Thus, the MAGL-COX-1 pathway mediates stress-induced brain PGE_2 increase, leading to depressive-like behavior. The resultant PGE_2 is involved in chronic stress attenuating the dopaminergic pathway to the medial prefrontal cortex via its receptor EP1 (Figure 3). As stress-induced prefrontal dopaminergic response and D1 receptor activation prevent the induction of depressive-like behavior (Shinohara et al., 2018), this PGE_2-EP1 action likely contributes to this behavior (Tanaka et al., 2012). Peripheral inflammation induced by LPS also causes EP1-mediated dopaminergic attenuation, leading to avoidance from an LPS-associated compartment (Fritz et al., 2016; Klawonn et al., 2021). Thus, different stressors activate the same inflammatory process for their adverse behavioral effects.

4.2. Brain region-dependent heterogeneity of neuroinflammation

The PGE_2 increase selectively occurs in subcortical brain areas for its pro-depressive effect, but not in cortical areas, including the prefrontal cortex, where proinflammatory cytokines take this role instead. Thus, microglia appear to exert pro-depressive effects via different proinflammatory effectors, depending on brain areas. Indeed, microglia show highly variable patterns of stress responses, depending on brain regions (Stratoulias et al., 2019). Even in naïve mice, microglia show heterogeneity in gene expression profiles across brain regions (Masuda et al., 2020; Tan et al., 2019), which may contribute to the diversity of stress-induced microglial responses. However, TLR2/4 is involved in the cytokine increase in the prefrontal cortex and the PGE_2 increase in subcortical regions (Nie et al., 2018; Nie et al., 2019). These findings suggest that TLR2/4 coordinate multiple inflammatory responses derived from microglial activation to promote depressive-like behavior. Unexpectedly, MAGL inhibition did not affect stress-induced elevated anxiety, despite the loss of stress-induced brain PGE_2 increase (Nie et al., 2019).

As elevated anxiety depends on the PGE_2-EP1 signaling, peripheral PGE_2 synthesis independent from MAGL could be involved (Figure 3). Thus, brain and peripheral inflammations could be preferentially involved in depressive- and anxiety-like behaviors, respectively.

4.3. Roles of astrocytes

Astrocytes are also involved in synaptic regulation and neuroinflammation. Under stress conditions, glial fibrillary acidic protein (GFAP), a marker of astrocytes, reportedly decreases with edematous changes in their processes, possibly affecting the BBB integrity (Tynan et al., 2013; Sántha et al., 2016). Pharmacological ablation of astrocytes in the prefrontal cortex recapitulates chronic unpredictable stress-induced depressive-like behaviors (Banasr & Duman, 2008), suggesting a pro-depressive role of stress-induced astrocytic dysfunctions. ATP release from astrocytes could exert antidepressant-like effects since blockade of astrocytic ATP release with various genetic methods causes depressive-like behavior (Cao et al., 2013). The antidepressant fluoxetine increases astrocytic ATP release, inducing astrocytic BDNF synthesis via purine receptors for its antidepressant-like effects (Kinoshita et al., 2018). Since astrocytes encapsulate and directly contact neuronal structures, including synapses, whether and how astrocytes interact with microglia under stress may provide a clue to the mechanism of neuroinflammation-induced neuronal remodeling.

5. Clinical implications of stress-induced immune responses

Like the above rodent studies, clinical studies have suggested that psychological stress induces innate immune responses in humans. For example, medical interns showed higher stress intensity and frequency while working on the intensive care unit (ICU) than off

duty (Heidt et al., 2014). While working in the ICU, circulating neutrophils and monocytes were increased, showing the association between psychological stress and leukocyte mobilization. The low socioeconomic status that may cause chronic social stress is associated with increased monocytes in the blood (Powell et al., 2013).

Stressful life events in adulthood and childhood maltreatment increase the risk of major depressive disorder (MDD), depending on genetic susceptibility (e.g., Caspi et al., 2003). Numerous papers have reported altered levels of cytokines and chemokines in depressive patients (Table 1) (Köhler et al., 2017; Milenkovic et al., 2019). Notably, these abnormality exists in drug-naïve patients (Syed et al., 2018). Consistent with stress-induced mobilization of myeloid cells, circulating neutrophils and monocytes increase in MDD patients (Hasselmann et al., 2018; Lynall et al., 2020). Increased platelets and decreased erythrocytes have also been reported (CAI et al., 2017), as seen in mice with repeated social defeat stress (Mckim et al., 2018). Circulating lymphocytes may also be affected in MDD patients (Toben et al., 2015; Zorrilla et al., 2001), although the direction and amplitude vary across studies. Nonetheless, the neutrophil/lymphocyte ratio correlates with the severity of depression, based on several independent studies (Özyurt et al., 2018; Sunbul et al., 2016). A recent study used multivariate analyses of multiple leukocyte subsets to stratify MDD patients (Lynall et al., 2020). In this study, a subgroup of depressive patients named the inflamed depression subgroup showed increased neutrophils, monocytes, CD4+ T cells, and C-reactive protein and IL-6. This subgroup had higher severity of depressive symptoms than the uninflamed remains. Thus, inflammation may not be involved in all depressive patients, as other studies have associated inflammation with atypical depression, suicidal ideation, and treatment resistance (Beurel et al., 2020). PET studies using the TSPO ligand [^{18}F]FEPPA have

suggested neuroinflammation in selective brain areas, including prefrontal cortices, that correlates with the severity and duration of depressive symptoms (Setiawan et al., 2015; Setiawan et al., 2018). Whether and how neuroinflammation is related to peripheral inflammation in the inflamed depression subgroup remains to be studied.

Clinical trials have tested the therapeutic effects of drugs targeting inflammatory molecules in MDD and other depressive patients. Consistent with the roles of PGE_2 in chronic stress-induced depressive- and anxiety-like behaviors (Tanaka et al., 2012; Nie et al., 2019), celecoxib, a non-steroidal anti-inflammatory drug that inhibits PG synthesis, significantly improves antidepressant effects of reboxetine (Müller et al., 2006). Meta-analyses have confirmed this celecoxib's effect for augmenting therapeutic effects of antidepressants (Na et al., 2014). Therapeutic effects of etanercept, a TNF inhibitor, for depression were initially discovered in psoriasis, as etanercept improved depressive symptoms in a manner little correlated to physical symptoms (Tyring et al., 2006). Later studies have shown similar therapeutic effects on depressive symptoms in other inflammatory diseases, such as Crohn's disease (Guloksuz et al., 2013). Given the association of inflammatory cytokines with treatment resistance, a clinical trial tested the therapeutic effects of infliximab, a TNF antagonist, in treatment-resistant depressive patients (Raison et al., 2013). Despite the lack of overall improvement, exploratory analyses suggested that infliximab improves depressive symptoms in patients with high baseline inflammatory markers.

Since the inflammatory status varies among depressive patients (Köhler-Forsberg et al., 2019), the inflammation-based stratification may help optimize antidepressant effects of anti-inflammatory drugs. As proposed by the rodent findings above, brain and peripheral inflammations may serve different symptoms of depressive patients. We

should characterize the effects of respective drugs on the brain and peripheral inflammations to establish personalized medicine targeting inflammation in depression.

6. Conclusions and future perspectives

In this review, we summarized rodent studies on the effects of stress on immune systems in the periphery and brain. Despite the complexity of stress-induced immune responses depending on stress and host conditions, studies have demonstrated crucial roles of these immune responses, at least in part, for depressive- and anxiety-like behaviors.

In the periphery, stress mobilizes innate and adaptive immune cells in distinct spatiotemporal manners. Neutrophils and monocytes egress from the bone marrow upon stress via concerted actions of sympathetic nerves and glucocorticoids and in a manner affected by gut microbiota. These immune responses in the periphery promote stress-induced depressive- and anxiety-like behaviors by infiltrating the brain, releasing cytokines, and affecting vascular endothelial functions. In the brain, innate immune receptors TLR2/4 activate microglia, which in turn secrete TNF-α and IL-1α in the medial prefrontal cortex and PGE_2 in subcortical brain areas, respectively. The former appears to alter the morphology and activity of prefrontal neurons, whereas the latter attenuates the dopaminergic projection to the medial prefrontal cortex. These actions promote depressive-like behavior in concert. Depressive patients have increased neutrophils and monocytes, which may represent stress pathology seen in rodent stress models. So far, drugs targeting PGE_2 and TNF-α have been tested for their therapeutic effects in MDD patients, yielding promising results. Therapeutic effects of these drugs could depend on the inflammation status of the patients, suggesting the need for inflammation-based

stratification to establish anti-inflammatory drugs for depression. The actions of anti-inflammatory drugs on brain and peripheral inflammations warrant investigations to decipher an optimal subgroup of depressive patients for each drug.

Although rodent studies have shown the behavioral effects of both brain and peripheral inflammations, whether brain and peripheral inflammations interact with each other or function separately remains unclear, and we should elucidate the mechanisms for the brain-periphery crosstalk. Autonomic and neuroendocrine systems mediate neural stress signals to peripheral inflammation. On the other hand, circulating leukocytes and their secreted substances integrate signals from multiple peripheral organs, such as the bone marrow, spleen, lymph nodes, and gut, to the brain parenchyma directly or indirectly. Alongside, myeloid cells could migrate directly from the skull bone marrow to the brain, skipping the entry to the circulation. Thus, the dynamics and actions of the respective systems under stress need to be precisely understood. This direction of research is essential from basic and clinical perspectives of stress-induced immune responses. It is even more so because neural and peripheral inflammation could play differential roles on depressive- and anxiety-like behaviors, respectively.

Epidemiological studies have claimed the gene x environment interaction in the pathology of mental illness, including depression. Although most studies have used inbred C57BL/6 mice to analyze stress-induced immune responses, several findings employed two mouse strains, C57BL/6 and BALB/c, that differ in stress susceptibility, implicating neutrophils in genetic stress susceptibility. Thus, stress-induced immune responses may offer a novel mechanism of the gene x environment interaction for depression. Finally, given specific neural circuits for respective stress-induced behaviors, how these immune responses influence neural circuits remains to be clarified, and this

understanding might be exploitable to translating rodent findings to clinics.

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Conflict of interest

Y.I. is an employee of Sumitomo Dainippon Pharma Co., Ltd.

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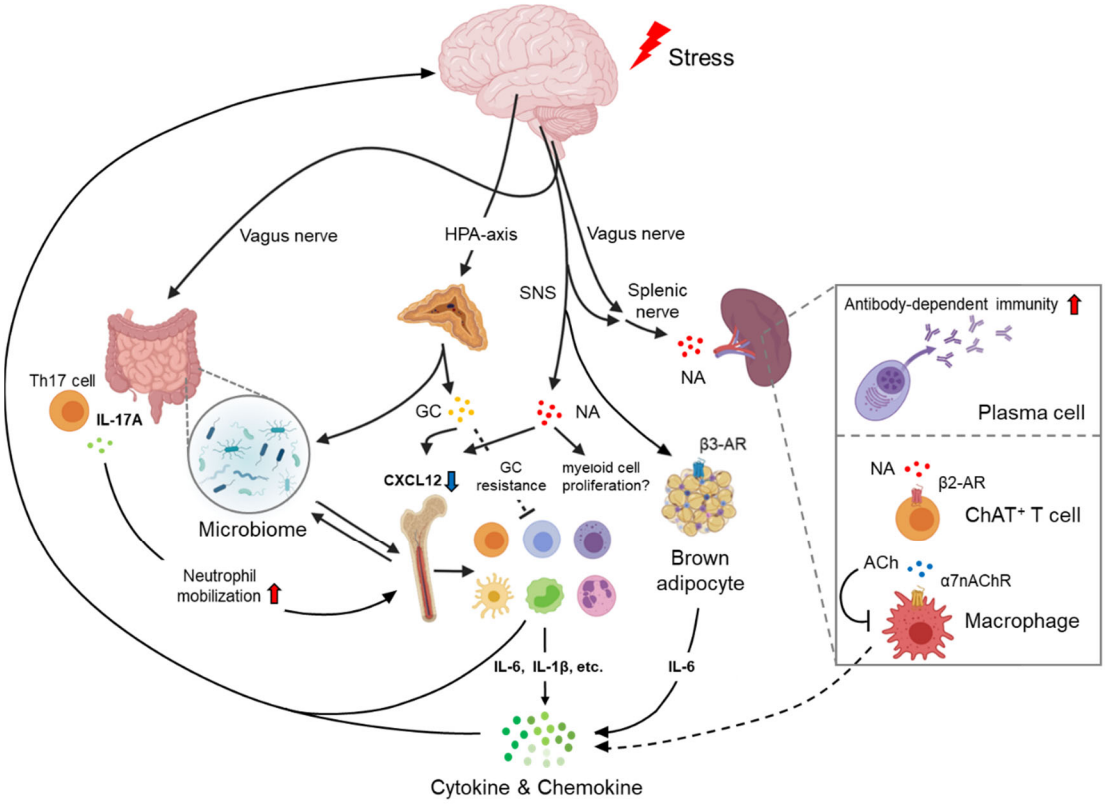

Figure 1. The impact of stress on immune systems in the periphery

Stress activates the sympathetic nervous system (SNS) and mobilizes myeloid cells, mainly monocytes and neutrophils, to the circulation via noradrenarline (NA) and adrenaline and their receptors. The hypothalamic-pituitary-adrenal (HPA) axis increases circulating glucocorticoids (GC) upon stress, contributing to myeloid cell mobilization as well. These effects are mediated by reduced expression of CXCL12 that retains myeloid cells in the bone marrow via CXCR4. Besides, SNS and GC have direct effects on leukocytes that are affected by chronic stress. Chronic stress induces glucocorticoid resistance in myeloid cells, thereby disinhibiting inflammatory responses. SNS could also promote myeloid cell proliferation. Stress-mobilized myeloid cells affect neural functions directly or indirectly via secretion of cytokines, such as IL-1β and IL-6, and chemokines (see Figure 2). A specific type of gut microbiota augments stress-induced neutrophil

mobilization likely via IL-17A secreted from intestinal Th17 cells. On the other hand, stress affects gut microbiota, perhaps via multiple routes, such as vagus nerves, glucocorticoids, leukocytes, and secreted cytokines. Stress affects immune cells in the spleen in multiple ways. Acute stress activates acetylcholine (ACh)-releasing $ChAT^{+}$ T cells by splenic sympathetic nerves and suppresses inflammatory responses in splenic macrophages via α7 nicotinic acetylcholine receptor (α7nAChR). Chronic stress could increase splenic plasma cells and enhance antibody-dependent immunity only when it is very mild and not accompanied by glucocorticoid release. Alongside, stress stimulates brown adipocytes via β3 adrenergic receptors (β3-AR), and the stimulated adipocytes secrete IL-6 into the circulation. Thus, the interactions among multiple organs, including the bone marrow, spleen, gut, and brown adipose tissue, shape stress-induced innate and adaptive immune responses.

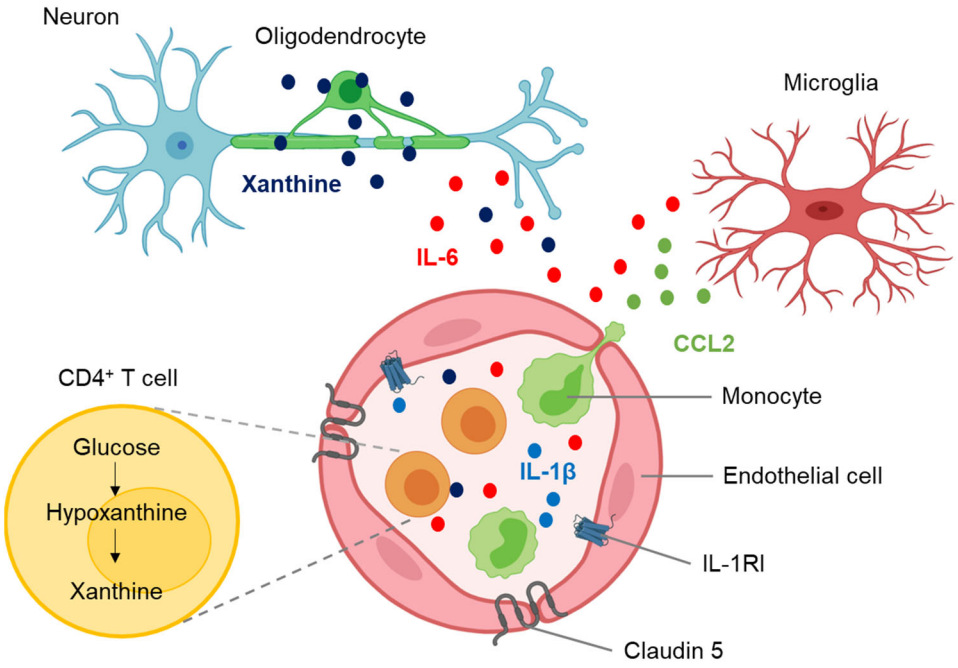

Figure 2. Stress signals from peripheral immune cells to the brain

Chronic stress induces CCL2 expression perhaps in microglia and astrocytes and attracts circulating monocytes to the brain vasculature via CCR2. Monocytes adhere to endothelial cells and further enter the brain perivascular space through the blood-brain barrier. Monocytes secrete proinflammatory cytokines, such as IL-1β and IL-6. IL-1β could act on IL-1RI on endothelial cells, disrupting their functions, such as the BBB integrity maintained by claudin-5, a tight junction protein. Through the disrupted BBB, circulating IL-6 infiltrates the brain parenchyma to alter neural circuit functions for emotional behaviors. Also, stress increases glycolysis and purine metabolism, which degrades purines to hypoxanthine and xanthine, in naïve $CD4^+$ T cells. These metabolic changes increases systemic levels of xanthine, which is proposed to activate oligodendrotes in the left amygdala via adenosine receptor A1 and induce anxiety-like behaviors.

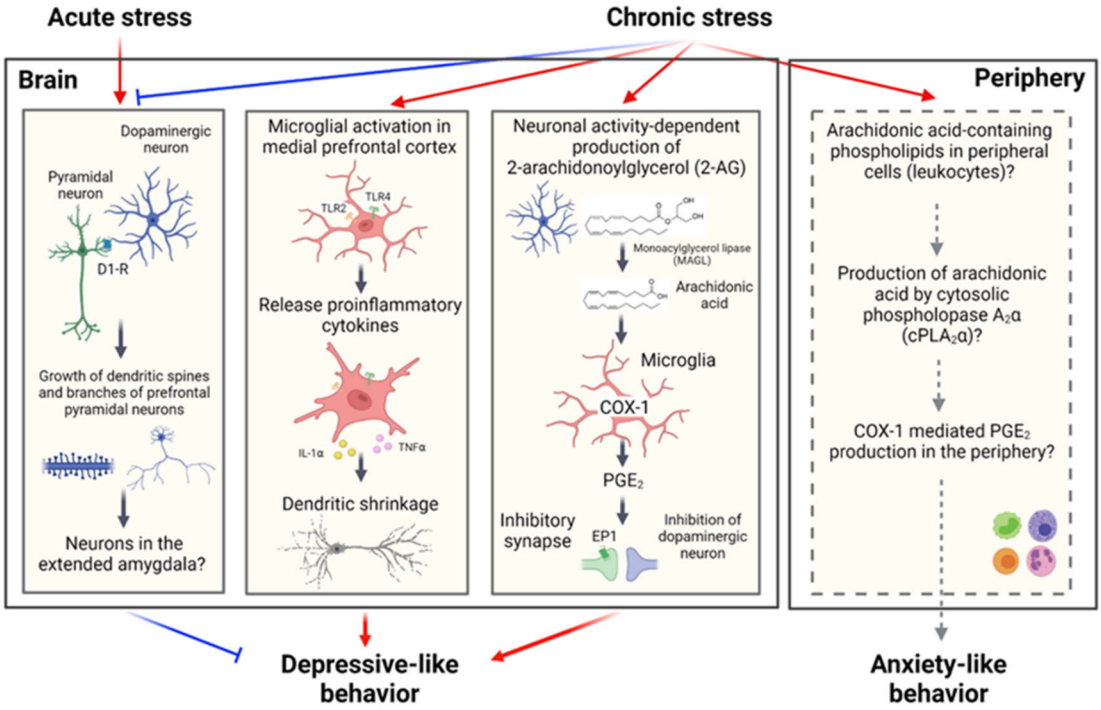

Figure 3. Multiple inflammatory pathways induced by stress for behavioral changes

The findings with a mouse model of social defeat stress demonstrate that chronic stress affects neuronal functions and behaviors via multiple inflammatory pathways. In the medial prefrontal cortex, chronic stress activates microglia via innate immune receptors TLR2/4. The activated microglia then secretes proinflammatory cytokines, TNF-α and IL-1α, leading to dendritic atrophy and attenuated neuronal activities. In subcortical regions, monoacylglycerol lipase (MAGL) metabolizes the endocannabinoid 2-AG produced from active neurons, and COX-1-expressing microglia converts the resultant arachidonic acid to PGE_2. This PGE_2 synthesis increases upon chronic stress in a TLR2/4-dependent manner and augments via its receptor EP1 synaptic inhibition to the mesoprefrontal dopaminergic pathway that is activated by acute stress for stress resilience. These neuroinflammatory pathways induced by stress promote depressive-like behavior in concert. On the other hand, peripheral PGE_2 synthesis, perhaps from arachidonic acid cleaved from phospholipids via cytosolic phospholipase A2α (cPLA2α) in leukocytes,

could be involved in anxiety-like behavior, since this behavior, being dependent on the PGE_2-EP1 pathway, remains intact with MAGL inhibition.

Cell type	Chronic stress model in mice	References	Depressive patients	References
Erythrocyte	Total count ↓ Erythropoiesis (bone marrow) ↓	McKim et al., 2018	Total count ↓ Red blood cell distribution width (RDW) ↑	Cai et al., 2017
Platelet	Total count & size ↑ Megakaryocyte (bone marrow) ↑	Sandrini et al., 2017	Total count ↑ Mean platelet volume (MPV) ↑	Cai et al., 2017
T cell	CD4+ T cell, CD8+ T cell & Treg↓ Proportion of Th17 (Spleen) ↑	Ishikawa et al., 2020 Ambrée et al., 2019	Count of Treg ↓ Count of Th17 cell ↑ Count of CD8+ T cell ↓ or n.s. Count of CD4+ T cell ↑ or n.s. Apoptosis ↑	Chen et al., 2011 Wu et al., 2017 Lynall et al., 2020 Toben et al., 2015
B cell	Total count ↓ Immature & mature B cells (bone marrow) ↓	Ishikawa et al., 2020	Proportion of transitional B cells ↓ Activated cells ↓	Syed et al., 2018 Ahmetspahic et al., 2017
NK cell	Total count ↓ Cytotoxicity (lung) ↓	Ishikawa et al., 2020 Hunzeker et al., 2004	Total proportion ↓ Cytotoxicity ↓	Park et al., 2015
Monocyte	Total count ↑ Ly6C^{high} monocyte ↑ Pro-inflammatory phenotype ↑ GC resistance (Spleen) ↑ Monocytopoiesis (bone marrow & Spleen) ↑	Ishikawa et al., 2020 Stark et al., 2001 Weber et al., 2017 McKim et al., 2018	Proportion of non-classical monocytes ↑ Activation state of classical monocytes ↑ Pro-inflammatory phenotype ↑	Nowak et al., 2019 Hasselmann et al., 2018
Neutrophil	Total count ↑ Granulopoiesis (bone marrow & Spleen) ↑	Ishikawa et al., 2020 McKim et al., 2018	Total count ↑	Lynall et al., 2020

Table 1. Changes in circulating blood cells in stressed mice and depressive patients