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Robert Dantzer
Lucile Capuron *Editors*

Inflammation-Associated Depression: Evidence, Mechanisms and Implications

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Inflammation-Associated Depression: Evidence, Mechanisms and Implications

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Preface

The willingness to associate depression with inflammation appears counterintuitive. Why should the immune system be activated in depressed individuals who are withdrawn from their social environment, show little interest in pleasurable events, and feel the heavy weight of hopelessness, helplessness, and even guilt on their shoulders? In addition, the very first descriptions of immunity in depressed subjects in the 1970s revealed a picture of immunosuppression, as indicated by the abnormally low cytotoxic activity of their natural killer cells and the decreased ability of their circulating lymphocytes to proliferate in response to nonspecific mitogens. However, further studies carried out in the 1980s and 1990s revealed a much more nuanced reality, with evidence of activation of innate immune cells and T cells. These observations coincided with the independent discovery that proinflammatory cytokines can act outside the immune system, and in particular in the brain, to cause fever, activate the hypothalamic–pituitary–adrenal axis, and induce behavioral alterations resembling some of the features of depression. Further research work in this field revealed that cytokine actions on the brain account for why we feel sick and behave in a sick way when we are ill. This discovery led to a change in paradigm, with sickness being seen as a normal response to infectious pathogens sensed by innate immune cells, in very much the same manner as fear is an adaptive response to dangers detected by our exteroceptive sensory organs. If the fear system can go awry and generate anxiety disorders, the same could be said of the sickness system, with depression being one possible example of a malfunctioning system.

The possibility that depression is nothing more than a sickness disorder took time to penetrate the field of biological psychiatry. The main reason for this was the predominance of the neurotransmitter theories of psychiatric disorders, with their exclusive reliance on hardwired, chemically coded neuronal networks. It was difficult to reconcile the serotoninergic theory of depression with the somewhat fuzzy picture of brain cytokines produced by the brain's innate immune cells acting to modify the neuronal environment rather than to directly affect neurotransmission. Within the last 20 years or so, the views have profoundly changed—not only because of the rapid progress in neuroscience and neuroimmunology but also

because of the inability of the pharmaceutical industry to generate innovative treatments in psychiatry.

In reviewing what we have learned during these last 20 years in the field of inflammation and depression, we do not pretend that inflammation explains all instances of major depressive disorders or that anti-inflammatory drugs represent the universal panacea for the treatment of depression. Our position is much more nuanced. We would like readers of this book to see the intrusion of inflammation in the field of depression as an example of the advantages, but also the limitations, of what a change in paradigm can bring to biological psychiatry. It is easy to pretend that bringing new players on stage can point to new targets for drug research and development; however, bringing new players on stage cannot suffice in and of itself if psychopathology remains frozen in its usual categorical description of symptoms, whatever their usefulness for diagnosis and treatment.

For new players to be useful, their function in the brain must be defined in terms of neurobehavioral phenotypes, the so-called research domain criteria (RDoC) launched by the US National Institute of Mental Health. We now know that inflammation plays a role in a variety of psychiatric disorders ranging from depression to autism spectrum disorders and including bipolar disorders and schizophrenia. This calls for a dimensional approach to more easily link inflammatory mediators to specific behaviors, symptoms, and brain circuits.

We must also recognize that inflammation is unlikely to have the same effect at different time points in the neurodevelopmental trajectory. There is evidence that the inflammatory machinery has been co-opted by the central nervous system during its evolution to organize its structure and function and, in particular, to mediate synaptic pruning. It is easy to speculate that this intrinsic property of the brain inflammatory machinery can be profoundly disturbed by inflammatory events occurring outside the brain, for instance, in the maternal environment. These events will not have the same impact as those occurring at adulthood, once the structure of the brain is less plastic. At the opposite end of the spectrum, aging and its association with a switch from the adaptive immune system to the innate immune system, the so-called inflammaging, set the stage for any inflammatory event to become much more powerful in its impact on brain structure and function. This becomes even more crucial when peripheral inflammation takes place in the context of a neurodegenerative process that has already recruited the immune machinery of the central nervous system.

Even if much remains to be done in the field of inflammation and depression, it is important to form a precise picture of what has been achieved since the intrusion of inflammation on the stage. The chapters that compose the book *Inflammation-Associated Depression: Evidence, Mechanisms, and Implications* aim to present in a comprehensive manner what we currently know about the role of inflammation in depression, the mechanisms that are involved, and how these mechanisms interact with classic neurochemically coded neuronal networks. The book also examines the possible usefulness of pharmacological and non-pharmacological interventions targeting various inflammatory processes. Each chapter is written by experts with a proven record of clinical and/or preclinical research in the field.

The first part of the book focuses on the evidence in favor of a relationship between inflammation and depression. In the first chapter, Krista Lanctot and her colleagues explore the available preclinical and clinical evidence that supports the concept of a relationship between inflammation and depression. Lucile Capuron and Nathalie Castanon then show that inflammation drives distinct neuropsychiatric domains involving separate pathophysiological pathways. Inflammation is an important factor in the pathophysiology of several physical illnesses, such as cardiovascular disease. It can therefore explain the comorbidity between depression and this disease, as discussed by Angelos Halaris.

The second part of the book presents our current knowledge of the mechanisms of action of inflammatory mediators on brain functions. Charlotte D'Mello and Mark Swain present what is known about the immune-to-brain communication pathways that mediate the propagation of inflammation from the periphery to the brain. Dietmar Fuchs and his colleagues describe the metabolic pathways that are altered by inflammation at the periphery and that ultimately affect neurotransmitter synthesis and function. Robert Dantzer shows how a role for these pathways in the pathophysiology of inflammation-induced depression has been demonstrated at the preclinical level in appropriate animal models. Depression is an important comorbid disorder of autoimmune diseases, and Christopher Pryce and Adriano Fontana discuss how it is possible to study the mechanism of this comorbidity, and in particular the role of clock genes, at the preclinical level. The possibility that psychosocial stressors induce mood disorders by recruiting inflammatory processes at the periphery that then propagate into the brain is described by John Sheridan and his colleagues, on the basis of their elegant work on repeated social defeat.

In an ideal world, there should be some overlap between the pathophysiological mechanisms that are triggered by inflammation and the alterations in neurotransmission that have already been identified as key players in the pathophysiology of depression. Ebrahim Haroon and Andrew Miller show how inflammation affects brain glutamatergic neurotransmission, and Jennifer Felger presents how this results in decreased dopaminergic neurotransmission.

Neuroimaging has been used successfully to trace the brain pathways that are affected by inflammation. Neil Harrison provides an excellent synthesis of this line of research, illustrating areas of convergence and divergence within the literature on major depressive disorders. Jonathan Savitz shows how it is possible to relate alterations in the structure and function of specific brain areas to the imbalance between neurotoxic and neuroprotective kynurenine metabolites, which is one of the metabolic hallmarks of inflammation-associated depression. How this can relate to suicidality is further discussed by Elena Bryleva and Lena Brundin.

The third and last part of the book presents the implications of what we have learned about the treatment of depression within the field of inflammation. Charles Raison appropriately moderates the enthusiasm of those who would like to treat depression with anti-inflammatory agents. He carefully points out that at the very best only a subpopulation of depressed patients is likely to benefit from it, and the means to identify this subpopulation still need to be delineated. Bernhard Baune reviews the literature on the possible antidepressant effects of nonsteroidal anti-

inflammatory drugs and makes the point that even in the best-case scenario, the lack of consideration of the temporal dynamics of depression does not allow much speculation about the usefulness of this modality of treatment. Because many nutrients are claimed to have anti-inflammatory properties, it is certainly worthwhile to reconsider their possible antidepressant activity within the context of the relationship between inflammation and depression. This is what Valeria Mondelli and Carmine Pariante, together with their collaborators, propose to do. This part ends with a chapter by Anindya Bhattacharya and Wayne Drevets, who show how it is possible to use what has been learned in the field of neuroinflammation to innovate and develop new therapeutics for the treatment of depression.

When navigating the book, readers will quickly notice redundancy between chapters. The editors have deliberately kept these redundancies in order to show that there is a common basis within the community of researchers working in the field of inflammation-associated depression, whatever their origins and degree of specialization. The differences are in what is done with this knowledge and the way it is applied to the understanding of depression and the quest for treatment.

As mentioned at the beginning of this preface, we have come a long way but our task is not yet finished. We do hope that what is presented and discussed in this book will not only help in understanding what has already been achieved but also stimulate further research.

Houston, USA — Robert Dantzer
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Contents

Evidence for Inflammation-Associated Depression

Celina S. Liu, Alexander Adibfar, Nathan Herrmann, Damien Gallagher, and Krista L. Lanctôt

Abstract This chapter explores the evidence supporting inflammation-associated depression. Data to date suggest a bidirectional relationship between inflammation and depression wherein one process can drive the other. A wealth of animal and clinical studies have demonstrated an association between concentrations of pro-inflammatory cytokines – specifically interleukin (IL)-1β, IL-6, and tumor necrosis factor-α – and depressive symptoms. There is also evidence that this pro-inflammatory state is accompanied by aberrant inflammation-related processes including platelet activation factor hyperactivity, oxidative and nitrosative stress, and damage to mitochondria. These complex and interrelated mechanisms can collectively contribute to negative neurobiological outcomes that may, in part, underlie the etiopathology of depression. Mounting evidence has shown a

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concomitant reduction in both depressive symptoms and pro-inflammatory cytokine concentrations following treatment with pharmacological anti-inflammatory interventions. Taken together, the reviewed preclinical and clinical findings may suggest the existence of a distinct inflammatory subtype of depression in which these patients exhibit unique biochemical and clinical features and may potentially experience improved clinical outcomes with inflammation-targeted pharmacotherapy.

Keywords Cell-mediated immune activation • Cytokines • Inflammation • Major depressive disorder • Mitochondrial dysfunction • Oxidative and nitrosative stress • Platelet activating factors

Contents

1 Introduction

Major depressive disorder (MDD) is a chronic illness adversely affecting mood and cognition that results in substantial impairments in quality of life and global functioning [1]. MDD has a lifetime prevalence of 10–15% [2] and it is universally classified among the most burdensome disorders [3]. Despite current treatment efforts, depression is the leading cause of disability for individuals aged 12–44 years, resulting in an estimated 400 million disability days per year and costing approximately $83.1 billion in 2000 in the USA [3]. A better understanding of additional mechanisms involved in depression may lead to the discovery of more treatment options. Depression has been characterized as a pathological state featuring elevated inflammation, with evidence citing increased concentrations of pro-inflammatory cytokines and cell-mediated immune (CMI) activation, as well as associated aberrant pathways involving platelet-activating factors, oxidative and nitrosative stress (O&NS), and mitochondrial dysfunction. This chapter reviews the preclinical evidence and focuses on the clinical findings supporting inflammation-associated depression.

2 Evidence from Animal Models

While experimental animal models of depression cannot substitute for clinical depression states in humans, they can mimic certain behavioural dimensions of depression, thus providing an informative tool to generate theories on the etiopathological basis of the disease. Specifically, rodents can be subjected to various stress paradigms intended to replicate stressful life events, which have been shown to be a major causal contributor to the onset of depression [4]. Although several depressive symptoms including feelings of worthlessness and suicidal ideation cannot be detected with animal models, exposing rodents to acute or chronic stressors can reliably give rise to anhedonic and amotivational behaviour [5–9]. Importantly, the behavioural changes induced by acute and chronic stress paradigms are not only etiologically but also pharmacologically relevant, as they can be reversed by chronic treatment with antidepressant medications [10, 11]. However, limitations include experimental simplification, insufficient inter-laboratory reliability, and use of male rodents almost exclusively. As such, those preclinical models overlook gender difference, an important consideration when evaluating depression, as women are twice as likely as men to experience depression [12] with an earlier onset, longer duration, and greater severity [13].

An abundance of preclinical evidence implicates inflammation in the pathogenesis of depression. Numerous animal studies have reported increased central and peripheral concentrations of pro-inflammatory cytokines – particularly interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α – and anti-inflammatory cytokines in rodents subjected to various stress paradigms, namely the chronic mild stress (CMS) [14–22], learned helplessness [23], social isolation [24, 25], social defeat [26–29], and olfactory bulbectomy (OB) [30, 31] models of depression. Beyond those correlative findings, studies using genetically modified rodent strains have yielded mechanistically informative evidence. Knockout mice for the IL-1 receptor type I or transgenic mice with overexpressed IL-1 receptor antagonist (IL-1Ra) did not exhibit the behavioural and inflammatory changes that were induced in wild-type mice subjected to a 5-week CMS protocol [18]. Courbaji et al. found that IL-6 knockout mice did not display the typical learned helplessness characterized by absence of escape behaviour upon repeated exposure to uncontrollable aversive stimuli, such as tail shocks [23]. The restraint stress paradigm, which confines rodents to tightly enclosed spaces to restrict their movement, increases forced swimming test and tail suspension test immobility duration in stressed mice compared to controls and antidepressant-treated mice [32]. Kaster et al. showed that restrained TNF-α receptor 1 knockout mice displayed antidepressant-like behaviour, reflected by significantly decreased immobility time and increased sucrose consumption, compared to stressed wild-type mice [33]. In an olfactory bulbectomy model of depression, in which bulbectomized rats display depressive-like behaviour that is normalized by chronic antidepressant treatment [30], the anti-inflammatory cyclo-oxygenase-2 inhibitor celecoxib was shown to reverse

behavioural changes in OB rats [31]. Taken together, those results provide compelling evidence suggesting the involvement of inflammation in depression.

IFN-α immunotherapy has also been shown to induce inflammation, T cell activation, as well as IDO activation, which researchers have associated with the onset of depressive symptoms [34]. On the other hand, several studies have shown that antidepressant use can suppress CMI activation and significantly reduce pro-inflammatory markers including Th-1-like cytokines IL-2, TNF-α, and IFN-γ [35–38] as well as increase the anti-inflammatory cytokine IL-10 [39–41]. Those results suggest that antidepressants have negative immunoregulatory effects through the inhibition of specific inflammatory markers and through the stimulation of IL-10 release. A recent study found a direct correlation between Th17 cells and depression sensitivity, reporting increased depressive-like behaviour following Th17 cell administration in two different murine models of depression [42]. Accordingly, mice with reduced Th17 cell development were less prone to develop depression, and treatment with anti-IL-17 antibodies concomitantly abrogated Th17 development and mitigated depressive-like behaviour [42]. Those results suggest that increased IL-17 concentrations may predispose to depressive behaviour, but the role of IL-17 in depression requires further investigation and support from clinical studies [43].

3 Evidence from Clinical Studies

Compared to the global point prevalence of MDD in the general population, which has been estimated to be 4.7% in a review of 116 prevalence studies [44], co-morbid MDD is more prevalent in inflammation-associated conditions and illnesses, such as asthma, arthritis, coronary artery disease, diabetes, and obesity (see Table 1). Inflammation-associated depression in particular is associated with greater persistence and severity, later age of onset, and reduced motivation [55–57].

Clinical studies have found a significant association between numerous inflammatory cytokines and depressive symptoms, citing bidirectional pathways between the brain and the immune, endocrine, and neurotransmitter systems [58]. Compared to non-depressed individuals, those with major depressive disorder (MDD) demonstrate increased concentrations of several peripheral and cerebrospinal fluid inflammatory markers including IL-1, C-reactive protein (CRP), and monocyte chemoattractant protein-1 [59–61]. Researchers have also reported that patients with MDD exhibit significantly increased concentrations of TNF-α compared to those of healthy controls [62–64]. In addition to those cross-sectional findings, there are ample longitudinal data supporting a relationship between inflammation and depression as evidenced by a recent systematic review and meta-analysis, which reported that higher concentrations of CRP and IL-6 were associated with an increased risk of subsequent depressive symptoms in a total of 18,527 participants [65]. Importantly, the authors found that this effect remained significant after adjusting for age and socio-demographic variables [65]. Evidence from

Table 1 Prevalence of co-morbid MDD with inflammatory conditions

Inflammatory condition	Reported prevalence based on Diagnostic and Statistical Manual of Mental Disorders criteria (*N*%)
Asthma	7.6 [45]; 9.0 [46]; 25.0 [47]
Arthritis	16.8 [48]
Coronary artery disease	20.0–40.0 [49]
Diabetes	8.7 [50]; 16.0 [51]; 18.0 [52]
Obesity	12.6 [53]; 18.0 [54]

observational studies has been complemented by a number of interventional studies assessing the inclusion of various anti-inflammatory agents in the pharmacological management of depressive symptoms (see Table 2).

3.1 Cytokines and Cell-Mediated Immune (CMI) Pathways Contribute to Inflammation and Depression

Inflammatory cytokines are cell-signaling proteins that mediate and regulate the immune response and can be generally divided into two subgroups: pro-inflammatory cytokines, namely IL-1β, IL-6, IL-8, and TNF-α; and the anti-inflammatory cytokines IL-1Ra, IL-4, IL-10, and transforming growth factor (TGF)-β1 [116–118]. Mounting evidence has given rise to the hypothesis that inflammatory cytokines play a key role in many of the etiopathological mechanisms underlying depression including hypothalamic-pituitary-adrenal (HPA) axis dysregulation [119–121]; alterations in the serotonergic [122–124], noradrenergic [125–127], dopaminergic [128–131], and glutamatergic systems [132, 133]; and impaired neuroplasticity [134, 135].

In addition to the previously described animal models of depression, converging lines of evidence collectively point to the intimate involvement of cytokines in MDD. It has been suggested that in response to infection, peripheral inflammation triggers a syndrome of "sickness behaviour" that shares many features with MDD including anhedonia and fatigue [136, 137]. Numerous longitudinal studies have demonstrated that exogenous administration of cytokines can precipitate depressive symptoms [138–142]. In a similar fashion, lipopolysaccharide endotoxin injections and vaccinations, which stimulate an immune response, can concomitantly increase pro-inflammatory cytokine concentrations and depressive symptoms [143–145]. Cytokine concentrations have also been cross-sectionally associated with depression risk in many co-morbid inflammatory conditions (Table 1). A previous meta-analysis undertaken by our group found that IL-6 and TNF-α concentrations were consistently raised in somatically healthy patients with MDD [146]. The clinical literature assessing peripheral inflammatory markers is now being complemented with direct measurements of CNS inflammatory activation as

Table 2 Pharmacological anti-inflammatory interventions in individuals with depressive symptoms

Pharmacological intervention	Dose	Study design and sample size (n)	Effect on depressive symptoms and/or inflammation
Positive results (significant effect)			
ω-3 fatty acids [66]	2 × (EPA (180 mg) + DHA (120 mg))/ 3 × daily or placebo	4-month RCT in hemodialysis patients ($n = 54$)	• ω-3 supplement lowered BDI scores significantly after 4 months of intervention • Serum ferritin level and IL-10 to IL-6 ratio showed significant changes in favour of ω-3 supplement • ω-3 treatment was a significant predictor of reduced serum CRP, ferritin, and iPTH levels, and increased IL-10 to IL-6 ratio
ω-3 fatty acids [67]	EPA (1 g/d), DHA (1 g/d), or placebo	8-week, two-site, double-blind RCT in MDD patients ($n = 154$)	• All three groups demonstrated statistically significant improvement in the HAM-D-17, QIDS-SR-16, and CGI-S
ω-3 fatty acids [68]	6 × (EPA (180 mg) + DHA (120 mg))/ daily or placebo	4-month RCT in hemodialysis patients ($n = 40$)	• Significantly lowered BDI score in the ω-3 group compared with the placebo group
ω-3 fatty acids [69]	EPA (1.67 g EPA + 0.16 g DHA/d), or DHA (1.55 g DHA + 0.40 g EPA/d), or LA (2.2 g/d)	6-month, double-blind RCT in individuals aged >65 years with MCI ($n = 50$)	• Compared with the LA group, GDS scores improved in the EPA and DHA groups • Verbal fluency improved in the DHA group
ω-3 fatty acids [70]	EPA + DHA (300 mg/d of each) or placebo	6-month, double-blind RCT in individuals with mild-to-moderate depression ($n = 66$)	• After adjusting for cholesterol, body mass index, and history of thyroid dysfunctions, a statistically significant difference was seen in GDS-15 scores between both groups • Treatment with ω-3 was clinically more effective in treating depression in comparison with the placebo
ω-3 fatty acids [71, 72]	EPA + DHA (2.5 g/d) or placebo	8-week and 2-month, double-blind RCTs in depressed females ($n = 46$)	• Mean GDS at 8 weeks and 2 months was significantly lowered only for the ω-3 group
ω-3 fatty acids [73]	EPA + DHA (2 g/d)	8-week treatment after single-blind placebo lead-in in menopausal women ($n = 20$)	• Significant decrease in MADRS scores with ω-3 treatment

(continued)

Table 2 (continued)

Pharmacological intervention	Dose	Study design and sample size (n)	Effect on depressive symptoms and/or inflammation
ω-3 fatty acids [74]	EPA + DHA (1.2 g/d) or placebo	8-week, double-blind, parallel-group RCT in individuals experiencing a major depressive episode ($n = 432$)	• ω-3 supplementation benefitted individuals with major depressive episode without co-morbid anxiety disorders. • Trend toward superiority of ω-3 supplementation over placebo in reducing depressive symptoms
ω-3 fatty acids [75]	EPA (1 g/d) or placebo	8-week, double-blind RCT in individuals with MDD ($n = 35$)	• Greater decrease in HAM-D-17 scores in EPA group compared to placebo
ω-3 fatty acids [76]	EPA + DHA (1,920 mg/d)	6-week, open-label study in children and adolescents with juvenile bipolar disorder ($n = 18$)	• Clinician ratings of mania and depression were significantly lower and global functioning was significantly higher after supplementation • Parent ratings of internalizing and externalizing behaviours were also significantly lower following supplementation
ω-3 fatty acids [77]	EPA + DHA (500 mg or 1,000 mg; based on tolerability) or placebo	16-week, double-blind RCT in children with depression ($n = 28$)	• Significant effects of ω-3 on depressive symptoms measured using the CDRS, CDI, and CGI
ω-3 fatty acids [78]	EPA + DHA (6.6 g/d) or placebo	8-week, double-blind RCT in individuals with MDD ($n = 28$)	• ω-3 group had a significantly decreased score on the 21-item HAM-D compared to those in the placebo group
ω-3 fatty acids adjunctive to current therapy [79]	Ethyl-EPA (2 g/d) or placebo	4-week, parallel group, double-blind RCT in individuals with MDD ($n = 20$)	• Significant benefits of the addition of the ω-3 compared with placebo were found by week 3 of treatment
ω-3 fatty acids adjunctive to standard therapy [80]	EPA + DHA (1 g/d) + standard therapy or standard therapy alone	1 month, randomized, non-placebo, single-centre, prospective trial in individuals with acute myocardial infarction ($n = 52$)	• Greater decrease in BDI, STAI-S, and harm/loss emotions in ω-3 group
ω-3 fatty acids adjunctive to fluoxetine [81]	EPA (1 g/d), fluoxetine (20 mg/d), or combination of both EPA + fluoxetine	8-week, double-blind RCT in individuals with MDD ($n = 60$)	• EPA + fluoxetine combination was significantly better than fluoxetine or EPA alone from the fourth week of treatment. • Fluoxetine and EPA appear to be equally effective in controlling depressive symptoms

(continued)

Table 2 (continued)

Pharmacological intervention	Dose	Study design and sample size (n)	Effect on depressive symptoms and/or inflammation
ω-3 fatty acids adjunctive to citalopram [82]	Citalopram with 2 × (EPA + DHA (1 g/d)) or placebo	9-week RCT in individuals with MDD ($n = 42$)	• Combination therapy demonstrated significantly greater improvement in HAM-D scores over time beginning at week 4
Curcumin [83]	2 × 500 mg/d or placebo	8-week, double-blind RCT in MDD patients ($n = 50$)	• Greater reductions in IDS-SR_{30} score in curcumin-treated individuals
Curcumin [84]	2 × 500 mg/d or placebo	8-week, double-blind RCT in MDD patients ($n = 56$)	• Curcumin and placebo were associated with improvements in IDS-SR_{30} total score from baseline to week 4 • Curcumin-treated individuals experienced greater improvement in several mood-related symptoms compared to placebo group from week 4 to 8
NSAIDs [85]	Ibuprofen (3 × 800 mg/d), naproxen (2 × 500 mg/d), celecoxib (200 mg/d), or placebo	Study pooled data from five post-approval trials; each trial at 6-week, multicenter, double-blinded, active-comparator, parallel-group RCT in osteoarthritis patients ($n = 1,497$)	• Multivariable regression analysis demonstrated a detectable effect in lowering PHQ-9 score in the ibuprofen, naproxen, and celecoxib groups • Logistic regression analysis demonstrated a trend towards significant treatment effect of all NSAIDs compared with placebo
ASA adjunctive to SSRIs [86]	ASA (160 mg/d) + current antidepressant treatment	4-week, open-label study in treatment-resistant MDD patients ($n = 24$)	• Combination associated with a 52.4% response rate • 43% remission in total sample; 82% remission in responder sample • Responder group showed significant improvements in HAM-D within week 1 and remained sustained until day 28
Celecoxib adjunctive to reboxetine [87]	Reboxetine (4–10 mg/d) + celecoxib (400 mg/d) or placebo	6-week, prospective, double-blind, add-on study in individuals suffering from an acute depressive episode ($n = 40$)	• Celecoxib group showed significantly greater improvement in scores of the HAM-D compared to the reboxetine-alone group
Celecoxib adjunctive to sertraline [88]	Sertraline (200 mg/d) + celecoxib (2 × 200 mg/d) or placebo	6-week, double-blind RCT in patients with MDD ($n = 40$)	• Celecoxib group showed significantly greater reduction in serum IL-6 concentrations and HAM-D scores compared to placebo group

(continued)

Table 2 (continued)

Pharmacological intervention	Dose	Study design and sample size (n)	Effect on depressive symptoms and/or inflammation
			• Celecoxib group experienced more response and remission than the placebo group • Significant correlation was observed between reduction of HAM-D scores and reduction of serum IL-6 levels at week 6
Celecoxib adjunctive to fluoxetine [89]	Fluoxetine (40 mg/d) + celecoxib (400 mg/d) or placebo	6-week, double-blind RCT in individuals with MDD ($n = 40$)	• Combination of fluoxetine and celecoxib showed a significant superiority over fluoxetine alone in the treatment of symptoms of major depression
Celecoxib adjunctive to current therapy [90]	Celecoxib (400 mg/d) or placebo	6-week, double-blind RCT in bipolar disorder individuals who were experiencing a depressive or mixed episode ($n = 28$)	• Celecoxib group had lower HAM-D scores after week 1 compared to placebo group • The improvement in week 1 of treatment was statistically significant when the analysis included only the subjects who completed the full 6-week trial
Infliximab [91]	Infliximab (5 mg/kg) administered at baseline, 2 weeks and 6 weeks	6-week longitudinal study in patients with ankylosing spondylitis ($n = 16$)	• Patients had depression scores above the cutoff value for both the HADS depression and BDI at baseline • Significantly lower BDI scores were found after first, second and third infusions of infliximab compared to initial score • Significant decrease in HADS appeared after second and third infusions
Infliximab [92]	Infliximab (5 mg/kg) administered at baseline, 2 weeks and 6 weeks	12-week double-blind RCT in patients with treatment-resistant depression ($n = 60$)	• Significant interaction between treatment, time, and log baseline hs-CRP concentration with change in HAM-D scores (baseline to week 12) • Baseline concentrations of TNF and its soluble receptors were significantly higher in infliximab-treated responders vs non-responders

(continued)

Table 2 (continued)

Pharmacological intervention	Dose	Study design and sample size (n)	Effect on depressive symptoms and/or inflammation
			• Infliximab-treated responders exhibited significantly greater decreases in hs-CRP from baseline to week 12 compared with placebo-treated responders
Equivocal results (some effect)			
ω-3 fatty acids [93]	2 × EPA (500 mg/d) or placebo	12-week RCT in diabetes mellitus individuals with MDD ($n = 25$)	• HPA-axis reactivity significantly decreased in the EPA-group but not in the placebo-group • EPA did not influence other oxidative stress, inflammatory, or one-carbon-cycle parameters compared to placebo
ω-3 fatty acids [94]	EPA + DHA + tocopherol (200 mg/d) or placebo	3-month, double-blind RCT in individuals with Parkinson's disease and MDD ($n = 29$)	• Individuals supplemented with ω-3 showed a significant decrease in MADRS and CGI-Depression scores • No difference among groups in BDI
ω-3 fatty acids [95]	DHA (Group A 1 g/d; Group B 2 g/d; Group C 4 g/d)	12-week, double-blind trial in individuals with MDD ($n = 35$)	• Groups A and B had significant decreases in HAM-D-17 scores • 0% completer response rate for Group C
ω-3 fatty acids [96]	Ethyl-EPA (1, 2, or 4 g/d) or placebo	12-week, double-blind RCT in individuals with persistent depression ($n = 60$)	• 1 g/d group showed a significantly better outcome than the placebo group on all 3 rating scales (BDI, HAM-D, MADRS) • 2 g/d group showed little evidence of efficacy, whereas the 4 g/d group showed non-significant trends toward improvement
ω-3 fatty acids adjunctive to current therapy [97]	EPA (1 g/d), DHA (1 g/d), or placebo	12-week, single-centre, double-blind, multi-arm, parallel-group RCT in individuals with mild to moderate depression ($n = 81$)	• EPA group showed a significantly lower mean HAM-D score at study endpoint compared with those in the DHA or placebo groups • Response to treatment (defined as a $\geq 50\%$ decrease from the baseline HAM-D score) was only observed in 6 individuals receiving EPA, while no one in any of DHA or placebo groups responded to treatment

(continued)

Table 2 (continued)

Pharmacological intervention	Dose	Study design and sample size (n)	Effect on depressive symptoms and/or inflammation
Curcumin adjunctive to antidepressants [98]	Antidepressants (escitalopram or venlafaxine) + 500 mg/d curcumin or placebo	5-week, double-blind RCT in individuals with first-episode depression ($n = 40$)	• Significant positive changes in both groups from baseline to the end of the study in all scales of measurement; no difference between curcumin and placebo • Individuals in the curcumin group demonstrated a trend to a more rapid relief of depressive symptoms in comparison with those in the placebo group
Aspirin adjunctive to citalopram	Aspirin (160 mg/d) + citalopram (20 mg/d)	Double-blind RCT in MDD patients ($n = 10$)	• 8/10 patients showed severe anxiety and akathisia from early days of this trial • Except for two patients, researchers discontinued the medication during 14 days of this trial • Three patients were hospitalized due to anxiety and akathisia • Two patients reported suicidal behaviour after the onset of this trial • Researchers suggest that this combination is not safe and there are some serious and intolerable adverse effects
Negative results (no significant effect/difference)			
ω-3 fatty acids [99]	8 × (DHA (2 g) + EPA (0.6 g) + Vitamin E (10 mg))/daily or placebo	16-week, double-blind RCT in MDD patients ($n = 95$)	• Mean changes in scores of depression did not differ significantly between the two groups • ANCOVA showed that in the ω-3 group there was a significant correlation between the change in erythrocyte DHA and the change in scores of depression
ω-3 fatty acids [100]	EPA (1.74 g) + DHA (0.25 g)/d or placebo	4-week, double-blind RCT in recovered depressed patients ($n = 71$)	• Small effects of ω-3 supplementation on aspects of emotional decision-making and on self-reported states of depression and tension • No significant effects were observed on memory, attention, cognitive reactivity, and depressive symptoms

(continued)

Table 2 (continued)

Pharmacological intervention	Dose	Study design and sample size (n)	Effect on depressive symptoms and/or inflammation
ω-3 fatty acids [101]	EPA + DHA (400 mg/d), or LA (2 g/d), or both EPA + DHA and LA, or placebo	Secondary analysis of double-blind RCT (Alpha Omega Trial), follow up at 40 months in individuals who experienced a myocardial infarction ($n = 4{,}116$)	• Depressive symptoms or dispositional optimism did not differ between groups with the use of ω-3 fatty acids compared with placebo at the 40-month follow-up
ω-3 fatty acids [102]	EPA + DHA (6 g/d) or placebo	6-week, double-blind RCT in women with perinatal depression ($n = 26$)	• No significant difference in depression scores between those receiving ω-3 and those receiving the placebo
ω-3 fatty acids [103]	EPA + DHA (2.8 mg/d) or placebo	4-month, double-blind RCT in MDD patients ($n = 83$)	• ω-3 supplementation provided no additional benefit to conventional treatment of depression
ω-3 fatty acids [104]	EPA + DHA (0.5 g/d, 1.4 g/d or 2.8 g/d)	8-week, dose-ranging RCT in individuals with postpartum depression ($n = 16$)	• All groups did not significantly differ in pre- or post-test scores, or change in scores using the EPDS and HAM-D
ω-3 fatty acids [105]	DHA (200 mg/d) or placebo	4-month, double-blind RCT in new mothers ($n = 138$)	• No difference between groups in either self-rating or diagnostic measures of depression
ω-3 fatty acids [106]	DHA (2 g/d) or placebo	6-week, double-blind RCT in individuals with MDD ($n = 36$)	• Response rates were 27.8% in the DHA group and 23.5% in the placebo group; the difference in response rates between groups did not reach statistical significance
ω-3 fatty acids [107]	Ethyl-EPA + DHA (1.2 g/d) or placebo	8-week, double-blind RCT ($n = 120$) in women with moderate to severe psychological distress	• Outcomes in the PGWB, HSCL-D-20, and the HAM-D-21 improved in both groups, but no significant differences were noted between them
ω-3 fatty acids [108]	Ethyl-EPA (1 g/d) or placebo	12-week, parallel-group RCT in diabetes mellitus patients with MDD ($n = 25$)	• Depressive symptoms significantly decreased over time, yet no significant differences were found between those treated with Ethyl-EPA compared to placebo
ω-3 fatty acids adjunctive to current therapy [109]	EPA + DHA (8 g/d) or placebo	12-week, double-blind RCT in individuals with depression ($n = 59$)	• No evidence that ω-3 improved mood when compared to the placebo

(continued)

Table 2 (continued)

Pharmacological intervention	Dose	Study design and sample size (n)	Effect on depressive symptoms and/or inflammation
ω-3 fatty acids adjunctive to current therapy [110]	EPA (6 g/d) or placebo	4-month RCT in individuals with bipolar depression and rapid cycling bipolar disorder ($n = 44$)	• No significant differences on any outcome measure (YMRS or IDS) between the EPA and placebo groups
ω-3 fatty acids adjunctive to current therapy [111]	Ethyl-EPA (1 g/d) or placebo	12-week RCT in diabetes mellitus individuals with MDD ($n = 25$)	• No effect of Ethyl-EPA on BDNF levels and changes in BDNF levels and depression severity were not significantly associated
ω-3 fatty acids and psychotherapy [81]	EPA + DHA (1.9 g/d) or placebo	8-week RCT in perinatal women with MDD ($n = 51$)	• Participants in both groups experienced significant decreases in EPDS and HAM-D scores from baseline • No benefit of ω-3 fatty acids over placebo
ω-3 fatty acids adjunctive to sertraline [112]	Sertraline (50 mg/d) + (EPA + DHA (2 g/d)) or placebo	10-week, double-blind RCT in depressed individuals with coronary heart disease ($n = 122$)	• No differences in weekly BDI-II scores, pre-post BDI-II scores, or HAM-D scores • Groups did not differ on predefined indicators of depression remission or response
ω-3 fatty acids adjunctive to sertraline [113]	Sertraline (50 mg/d) and EPA + DHA (2 g/d) or placebo	Secondary analysis of 10-week clinical trial in individuals with coronary heart disease and MDD ($n = 122$)	• Baseline levels of hs-CRP, IL-6, and TNF-α were not associated with the 10-week post-treatment depression score • Treatment responders (>50% reduction from baseline BDI-II score) did not differ from non-responders in baseline hs-CRP, IL-6, or TNF-α • Depression remitters (BDI-II ≤8 at post-treatment) did not differ from non-remitters on the three baseline inflammation markers
Curcumin [114]	Fluoxetine (20 mg/d), curcumin (1,000 mg/d), or combination	6-week RCT in MDD patients ($n = 60$)	• As measured by the HAM-D, response rates were 77.8% in the combination group, 64.7% in the fluoxetine group, and 62.5% in the curcumin group • Mean change in HAM-D17 score at the end of trial was comparable in all groups

(continued)

Table 2 (continued)

Pharmacological intervention	Dose	Study design and sample size (*n*)	Effect on depressive symptoms and/or inflammation
NSAIDs [115]	Celecoxib (2 × 200 mg/d), naproxen (2 × 220 mg/d), or placebo	18-month, double-blind RCT across six sites in cognitively normal volunteers age 70 or older with a family history of Alzheimer-like dementia ($n = 2{,}312$)	• Mean GDS score, and the percentage with significant depressive symptoms, remained similar over time across all three treatment groups • No treatment effect on GDS scores over time in the subgroup of participants with significant depressive symptoms at baseline

ANCOVA analysis of covariance, *ASA* acetylsalicylic acid, *BDI* Beck Depression Inventory, *CDI* Children's Depression Inventory, *CDRS* Children's Depression Rating Scale, *CGI* Clinical Global Impression, *CGI-S* Clinical Global Impression-Severity Scale, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *EPDS* Edinburgh Postnatal Depression Scale, *g/d* grams/day, *GDS* Geriatric Depression Scale, *HADS* Hospital Anxiety and Depression Scale, *HAM-D* Hamilton Rating Scale for Depression, *HSCL-D-20* 20-item Hopkins Symptom Checklist Depression Scale, *Hs-CRP* High-sensitivity C-reactive Protein, *IDS-SR*$_{30}$ Inventory of Depressive Symptomatology Self-rated Version, *IFN-α* interferon-alpha, *IL* interleukin, *iPTH* intact parathyroid hormone, *LA* linoleic acid, *MADRS* Montgomery-Åsberg Depression Rating Scale, *MDD* Major Depressive Disorder, *mg/d* milligrams/day, *PGWB* Psychological General Well-Being Schedule, *PHQ* Patient Health Questionnaire, *QIDS-SR-16* 16-item Quick Inventory of Depressive Symptomatology-Self Report, *RCT* randomized controlled trial, *SSRI* selective serotonin reuptake inhibitor, *STAI-S* State-Trait Anxiety Inventory in a Specific Situation, *TNF-α* tumor necrosis factor-alpha, *ω-3* omega-3

Bold, italic, and bold-italic columns represent studies with positive results (significant effect), equivocal results (some effect), and negative results (no significant effect/difference) respectively

evidenced by a recent study using positron emission tomography, which found that MDD patients undergoing major depressive episodes featured elevated translocator protein density, a marker of microglial activation and neuroinflammation, in numerous brain regions compared to controls [147].

Research has also suggested that depression may partly result from cell-mediated immune (CMI) activation and inflammation (see Table 3) [164]. CMI activation is a component of the immune system involved in the interactions between immune cells [164]. Two recent meta-analyses [146, 165] have highlighted several inflammatory changes related to CMI activation in depression including an increase in the levels of plasma soluble IL-2 receptor (sIL-2R) and soluble CD8 (sCD8) molecule [148], the numbers of percentages of T cells [148], and the stimulated production of IFN-γ [39]. Clinical findings have also shown that depressed individuals, compared to controls, exhibit a higher level of serum neopterin [149], IL-6, acute phase proteins [150] and increased levels of kynurenine metabolites along the indoleamine 2,3-dioxygenase (IDO) pathway [151]. Activation of the IDO pathway contributes to lower plasma tryptophan, a consistently reported feature in depression [152]. Neopterin has also been recently implicated as a predictor for the development of depression in post-stroke individuals

Table 3 Evidence for putative inflammatory-related mechanisms associated with depression

Mechanism	Findings from clinical literature	Reference
Cell-mediated immune activation	CMI activation is associated with an increase in the concentrations of plasma sIL-2R and sCD8 molecule as well as an increase in the numbers of percentages of T cells	[148]
	CMI activation is associated with an increase in the stimulated production of IFN-γ	[39]
	Increased concentrations of serum neopterin in depressed patients compared to controls	[149]
	Increased concentrations of IL-6 and acute phase proteins in depressed patients compared to controls	[150]
	Increased levels of kynurenine metabolites along the IDO pathway	[151]
	Activation of the IDO pathway contributes to lower plasma tryptophan, which is a feature consistently reported in depression	[152]
	Neopterin is implicated as a predictive marker for the development of depression in post-stroke individuals	[153]
Oxidative and nitrosative stress	Imbalanced levels of ROS/RNS lead to O&NS	[154]
	Increased serum concentrations of the ROS peroxide and pro-oxidant enzymes, xanthine oxidase and monoamine oxidase in depressed patients compared to controls	[155]
	Increased lipid peroxidation by-products, malondialdehyde and 8-isoprostane, in depressed patients compared to controls	[156]
	Depression impairs antioxidant defenses resulting in lower serum total antioxidant capacity and lower serum concentrations of the enzymatic antioxidant paraoxinase 1 as well as the non-enzymatic antioxidants uric acid, albumin, high-density lipoprotein cholesterol, and zinc	
	Increased concentrations of 8-oxo-2′-deoxyguanosine, a marker of oxidative DNA damage, in depressed patients compared to controls	[157]
Mitochondrial dysfunction	Mitochondrial dysfunction includes disturbances in mitochondrial enzyme activity, increased production of ROS, increased mtDNA mutations or deletions, dysregulated calcium signaling, as well as impaired energy metabolism	[158]
	Reduced gene expression of mtDNA-encoded transcripts in depressed patients compared to controls	[159, 160]
	Reduced energy ATP production in depressed patients compared to controls	[161]
	Greater mitochondrial oxidative damage in depressed patients compared to controls	[162]
	Patients with IBD show reduced ATP levels within the intestine	[163]

ATP adenosine triphosphate, *CMI activation* cell-mediated immune activation, *IBD* inflammatory bowel disorder, *IDO* indoleamine 2,3-dioxygenase, *IFN-γ* interferon gamma, *IL-6* interleukin 6, *mtDNA* mitochondrial DNA, *O&NS* oxidative and nitrosative stress, *RNS* reactive nitrogen species, *ROS* reactive oxygen species, *sCD8* soluble CD8, *sIL-2R* soluble interleukin 2 receptor

[153]. In line with this finding, several studies have suggested that the high comorbidity rate between depression and inflammatory disorders may be, in part, explained by shared mechanisms including increased levels of pro-inflammatory cytokines and kynurenine metabolites [166].

3.2 *Platelet-Activating Factors (PAFs) and Oxidative and Nitrosative Stress (O&NS) Contribute to Inflammation and Depression*

The platelet-activating factor (PAF) family of pro-inflammatory phospholipids may be of particular relevance to several mechanisms thought to underlie depression including immune activation and oxidative stress [167]. Pro-inflammatory cytokines have been shown to initiate the PAF synthesis cascade [168]. Conversely, PAFs potentiate the immune response by stimulating the inflammatory eicosanoid cascade [169] as well as the release of pro-inflammatory cytokines IL-6 [170] and TNF-α [171]. A recent study conducted by Mazereeuw et al. found that greater plasma concentrations of the PAFs phosphocholine (PC)(*O*-12:0/2:0), PC(*O*-14:1/2:0), PC(*O*-17:3/2:0), and PC(*O*-18:3/2:0) were significantly associated with depressive symptom severity in a cohort of 26 patients with coronary artery disease [172]. Those preliminary findings support further investigation into PAF species as lipidomic biomarkers that may clarify the etiopathology of inflammation-associated depression.

Several primary studies and comprehensive reviews (see Table 3) have suggested that a dysregulation in oxidative and nitrosative (O&NS) pathways contributes to depression. O&NS refers to an imbalance in which the cytotoxic actions of reactive oxygen species (ROS) and reactive nitrogen species (RNS), highly unstable compounds that disrupt cellular signaling and damage nearby cellular macromolecules when present in excess [173], overwhelm the reparative functions of endogenous and exogenous antioxidants, which normally counteract the effects of ROS/RNS-induced toxicity [154]. This emerging mechanism is implicated in inflammation as well as other potential etiopathological components of depression such as lipid signaling and monoamine regulation [152, 174, 175]. In regard to inflammation, it has been shown that depression features autoimmune responses directed against immunogenic neoepitopes generated by ROS/RNS [174, 176, 177].

As ROS/RNS give rise to multiple products due to their reactivity with proteins, lipids, and nucleic acids [178], many clinical studies have assessed biomarkers of O&NS in relation to depression. Compared to controls, depressed patients have demonstrated consistently higher serum concentrations of the ROS peroxide [155], pro-oxidant enzymes, xanthine oxidase and monoamine oxidase [155], lipid peroxidation by-products, malondialdehyde and 8-isoprostane [156], and 8-oxo-2-′-deoxyguanosine, a marker of oxidative DNA damage [157]. Depression is also

characterized by impaired antioxidant defenses as evidenced by a recent meta-analysis that found lower serum total antioxidant capacity and lower serum concentrations of the enzymatic antioxidant paraoxinase 1 as well as the non-enzymatic antioxidants uric acid, albumin, high-density lipoprotein cholesterol, and zinc in patients with MDD compared to controls [156]. Normalization of many of these pro-oxidant and antioxidant markers with antidepressant treatment suggests that O&NS mechanisms may be particularly important to the pathophysiology and prognosis of inflammation-associated depression [155, 156, 179, 180].

3.3 Damage to Mitochondria and Mitochondrial DNA Contribute to Inflammation and Depression

Mitochondrial dysfunction includes disturbances in mitochondrial enzyme activity, increased production of ROS, increased mitochondrial DNA (mtDNA) mutations or deletions, dysregulated calcium signaling, as well as impaired energy metabolism [158]. Although most of the evidence implicating mitochondrial damage in inflammation-associated depression is derived from animal studies, a few clinical studies have suggested that mitochondrial dysfunction is prevalent in both inflammatory conditions [181] and MDD (see Table 3), as well as other psychiatric disorders such as schizophrenia and bipolar disorder [159]. Studies have reported that depressed patients show decreased gene expression of mtDNA-encoded transcripts [159, 160] and reduced energy (adenosine triphosphate (ATP)) production [161]. In addition, MDD patients demonstrated significantly greater mitochondrial oxidative damage when compared to a non-psychiatric cohort [162]. Similarly, patients with inflammatory diseases such as inflammatory bowel disorder (IBD) show mitochondrial dysfunction, including reduced ATP levels within the intestine [163]. More clinical studies are needed to further elucidate the role of mitochondrial dysfunction in the development and progression of inflammation-associated depression.

4 Discussion

Although there is ample mechanistic and clinical evidence implicating inflammation in depression, there is also evidence against the inflammation-depression hypothesis. For example, not all rodent models have exhibited depressive-like behaviours in response to neuroimmune activation [182] and not all depressed patients have consistently elevated inflammatory cytokine profiles [183–186]. A study undertaken by Lotrich et al. found that exogenous administration of IFN-α did not precipitate a major depressive episode in the majority of patients [187]. Those findings collectively suggest that there may be a subtype of MDD patients with an

elevated inflammatory status that gives rise to unique variations in both etiopathology and clinical presentation. In keeping with this theory, post-hoc analyses of a clinical trial conducted by Raison et al. indicated that treatment-resistant MDD patients with pre-existing elevations in CRP, TNF-α, and TNF soluble receptor concentrations experienced improved mood outcomes when treated with infliximab, a TNF-α antagonist [92].

Moreover, research suggests that anti-inflammatory treatment may also be efficacious in the prevention of depressive symptoms. For example, IFN-α therapy for chronic hepatitis C virus infection is frequently associated with depression and a recent randomized, controlled trial found that ω-3 fatty acid supplementation significantly delayed the onset of IFN-induced depression [134]. Other interventions, including exercise and psychotherapy, have also demonstrated a concomitant reduction in inflammatory cytokine concentrations and depressive symptoms. Rethorst et al. found a significant positive correlation between changes in IL-1β and changes in depression symptoms over a 12-week exercise period [135]. Furthermore, Thornton et al. also found that psychotherapy significantly reduced depressive symptoms and inflammatory marker concentrations in breast cancer patients. In addition, the authors stated that the effect of psychotherapy on inflammation was mediated by its effect on depressive symptoms [136].

It may also be more difficult for those with inflammation-associated depression to respond to conventional forms of antidepressant therapy, such as selective serotonin reuptake inhibitors (SSRIs). Fornaro et al. found that compared to early responders taking duloxetine, non-responders exhibited elevated inflammatory cytokine concentrations [188]. Inflammation may also vary among depressive subtypes as indicated by a recent study, which found that patients with atypical depression had higher serum CRP, IL-6, and TNF-α concentrations compared to those with melancholic depression [189]. Future research efforts should aim to better characterize depressive syndromes with an inflammatory contribution, understand their underlying mechanisms, and determine the effects of existing treatments, both pharmacological and non-pharmacological, on inflammation. More randomized controlled trials as well as longitudinal studies are required to evaluate both the acute and long-term benefits of combination therapy. Characterizing MDD patients with an underlying elevated inflammatory profile may ultimately help healthcare professionals develop a more effective personalized treatment plan for treatment-resistant individuals.

5 Conclusion

In brief, MDD is a complex pathophysiological state associated with excess inflammation. This appears to be driven by elevated pro-inflammatory cytokine concentrations and dysregulated CMI activation, as well as various interrelated inflammation-related mechanisms including PAF activity, O&NS, and mitochondrial dysfunction. The current state of knowledge driving the management of MDD

remains incomplete, but there is a growing body of preclinical and clinical evidence supporting the intimate involvement of inflammation in depression. Recent findings collectively suggest that there may be a specific subtype of depression that features an elevated inflammatory status. Further research aiming to better characterize the relationship between inflammation and depression may provide useful insights into both the etiopathology of depression and targeted treatments for this MDD subgroup that has shown to be resistant to conventional antidepressant pharmacotherapy.

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Role of Inflammation in the Development of Neuropsychiatric Symptom Domains: Evidence and Mechanisms

Lucile Capuron and Nathalie Castanon

Abstract The finding that inflammatory markers are elevated in various neuropsychiatric disorders raises the need of identifying the precise research domain criteria driven by inflammation. Based on the model of inflammation-induced depression it has been possible to identify distinct pathophysiological pathways leading to alterations in neurotransmitter metabolism with specific relevance for the development of symptom constellations that are common to various neuropsychiatric and neurodegenerative conditions. Moreover, converging data indicate that these pathways interact with relevant vulnerability factors and modulatory systems to ultimately impact the presentation of inflammation-driven neuropsychiatric symptoms. Altogether, these findings make inflammation a key pivotal factor in psychopathology. Developing treatments that target inflammation and modulate the pathways and systems by which inflammatory processes selectively affect brain function will be of particular relevance for the treatment of specific neurobehavioral symptom domains.

Keywords Cytokines • Depression • Inflammation • Neuropsychiatric symptom domains • Pathophysiology • Research domain criteria • Vulnerability

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Contents

1 Inflammation and Neuropsychiatric Symptoms

1.1 From Sickness to Neuropsychiatric Disorders

A large set of data documents the role of inflammation in the development of neuropsychiatric symptoms. Over the last decades, clinical and experimental investigations focusing on the intricate relationship between the innate immune system and the brain have supported a pivotal role for cytokines in the induction of both sickness behavior and neuropsychiatric symptoms [1–3]. Most cytokines are produced locally by activated innate immune cells in response to tissue injury, infection, or inflammation. They are also able to act systemically on distant organs, including the brain. Activation of immune-to-brain pathways by peripheral cytokines ultimately induces the transient production of brain cytokines by activated endothelial and glial cells, particularly microglia [4, 5]. By in turn altering neurotransmitter metabolism and function, neuroendocrine activity, neural plasticity, and/or brain circuitry, brain cytokines coordinate behavioral changes collectively referred to as sickness behavior [1, 6]. Necessary for infection recovery, sickness behavior usually resolves within a few days, once microbial pathogens have been cleared and the innate immune system is no longer activated. However, failure to tightly regulate systemic immune activation and/or brain microglial activation leads to a significant and prolonged induction of peripheral and brain cytokines. Such sustained inflammation might in turn culminate in neuropsychiatric symptoms, particularly when it ultimately affects neurotransmitter systems playing a key role in mood regulation and/or cognitive function [2, 7–9]. In support of this notion, a substantial number of clinical and preclinical studies have clearly established the involvement of cytokines in the pathophysiology of depression (see for review [1–3] and the other chapters in this book). The most compelling evidence for this comes from findings obtained with the model of cytokine-induced depression in medically ill patients undergoing cytokine immune therapy. Using this model, we and others have shown that 30–50% of patients free of any psychiatric antecedent but treated chronically with interferon (IFN)-alpha develop major depression during the course of the treatment and that this effect can be prevented by prophylactic administration of antidepressants [10–12].

1.2 Towards the Identification of Common Major Symptom Domains Targeted by Inflammatory Processes

While the role of inflammation in the development of neuropsychiatric disorders has been most extensively studied in major depression, a number of recent reports show that inflammatory processes are also activated in bipolar depression, anxiety disorders, autism, schizophrenia, cognitive decline, and neurodegenerative diseases [13–19]. In most of these conditions, the biomarkers that have been commonly found to be dysregulated (most often in the direction of an upregulation) include the pro-inflammatory cytokines interleukin [IL]-1, IL-6, IL-12, tumor necrosis factor [TNF]-α and interferon [IFN]-γ and some of their soluble receptors, the cytokine soluble receptors, TNF-R1 and 2, and the anti-inflammatory cytokines, IL-10 and IL-4, the chemokines, eotaxin, and the acute phase protein, C reactive protein.

The non-specific association found between inflammation and neuropsychiatric disorders and the identification of common reliable inflammatory markers across those different disorders indicate that inflammatory processes could play a role in the etiology of symptom dimensions or symptom domains that overlap in – or are shared by – those conditions. This scenario is consistent with the Research Domain Criteria (RDoC) framework, which aims at improving the classification of mental disorders by incorporating multiple behavioral and neurobiological domains/dimensions cut across diagnostic categories [20]. Furthermore, it gives inflammation a distinctive and strategic position in this framework.

1.3 Evidence for a Role of Inflammation in Distinct Neuropsychiatric Domains

The notion that inflammation can contribute to the pathophysiology of multiple neuropsychiatric symptom dimensions emerged from studies of cytokine-induced depression. Patients treated with IFN-α develop two different sets of symptoms with differential phenomenology and antidepressant treatment responsiveness. The first set is characterized by symptoms of fatigue, lack of energy (anergia), lassitude, decreased motivation, motor slowing, reduced appetite, and altered sleep. It arises during the first weeks of IFN-α treatment in nearly all patients. This symptom constellation is not prevented, or only poorly, by the prophylactic administration of a selective serotonin reuptake inhibitor (SSRI) antidepressant such as paroxetine. The second set of symptoms includes sadness, decreased mood, reduced ability to experience pleasure (anhedonia), and impaired cognitive function. It develops usually at later phases of IFN-α treatment and it does so only in a subpopulation of patients (30–50% of patients), probably because of vulnerability factors. In contrast to the first set of symptoms, this second set of symptoms is prevented by SSRI antidepressant pretreatment (Fig. 1). This distinct phenomenology of cytokine-induced symptoms of depression attests of the involvement of separate

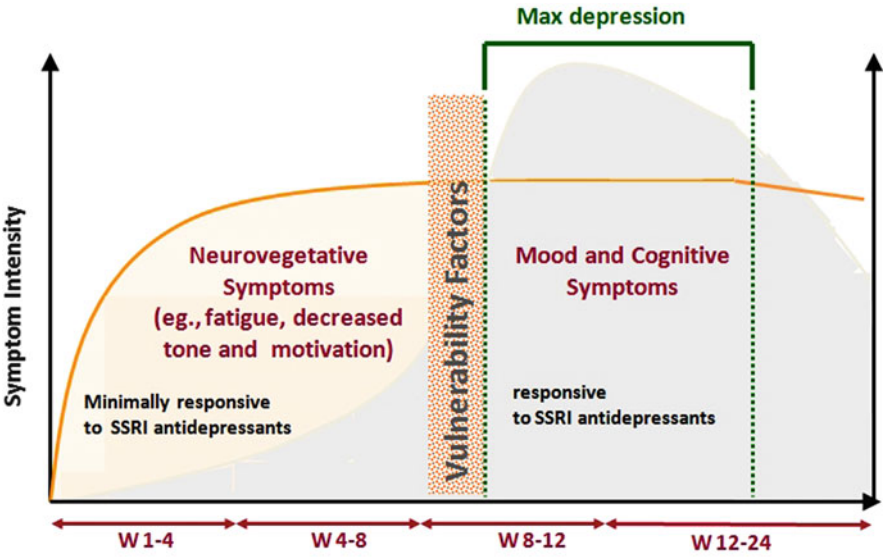

Fig. 1 Biphasic onset of interferon (IFN)-α-induced neuropsychiatric symptoms. IFN-α treatment in medically ill patients induces two types of neuropsychiatric symptom dimensions. Neurovegetative symptoms, including fatigue, psychomotor slowing, decreased tone and motivation, reduced appetite, and sleep disturbance develop early in the largest proportion of patients. These symptoms remain present during the duration of treatment and are minimally responsive to the prophylactic administration of the antidepressant, paroxetine, a selective serotonin reuptake inhibitor. Mood and cognitive symptoms, including depressed mood, anxiety symptoms, and cognitive disturbance, develop at later stages of treatment in 30–50% of patients, suggesting the existence of vulnerability factors. These symptoms can be prevented by the administration of paroxetine. (Adapted from Capuron and Miller [2])

underlying mechanisms. Interestingly, those mechanisms are likely to be involved in the physiopathology of the multiple neuropsychiatric disorders in which those core neurobehavioral symptom domains are also expressed and in which activated inflammatory processes have been reported.

Animal models have been developed to further assess the causal role of inflammation in the development of these distinct symptom constellations and to identify potential therapeutic targets. First attempts to experimentally assess the neurobiological bases of inflammation-induced neuropsychiatric symptoms have used acute immune activation by recombinant inflammatory cytokines or cytokine inducers (bacterial lipopolysaccharide: LPS and viral mimetic poly I:C) [21–23]. However, the results obtained using this model can be biased by the profound lethargy and motor impairment occurring in the first hours following immune stimulation [22, 24]. More suitable paradigms have implemented experimental designs based on the clinical evidence that emotional and cognitive symptoms develop later than sickness and neurovegetative symptoms in cytokine-treated patients [11]. Accordingly, it has been possible to differentiate the initial phase of decreased motor activity that progressively returns to normal from the delayed depressive-like

behaviors that become visible after sickness has dissipated and remain up to 24 h after LPS administration to mice [25–27]. A similar dissociation between LPS-induced sickness behavior and increased anxiety-like behavior [28], or impaired spatial memory [8], has been also reported using the same experimental design. The brain structures that underlie LPS-induced sickness behavior differ from those responsible for depressive-like behavior, strongly supporting the notion emerging from clinical studies that different mechanisms and neurobiological substrates are involved [25]. Similarly, mice inoculated with Bacillus Calmette-Guerin (BCG), which chronically activates the immune system [29], also display a transient episode of sickness behavior (lasting for a few days) followed by a longer occurrence of depressive-like behavior (up to several weeks) [30–32].

2 Inflammation and the Specificity of Neuropsychiatric Symptoms Units

2.1 A Multimodal Biological Substrate

The findings that cytokines are responsible for the expression of distinct neurobehavioral symptom dimensions that are expressed across multiple neuropsychiatric conditions prompted a surge of interest for the identification of the biological substrates that are differentially targeted by cytokines and that may selectively underlie those dimensions. In that context, the impact of inflammation on the serotoninergic and glutamatergic systems might be of particular relevance for the development of mood and cognitive symptoms whereas inflammation-induced alterations in dopamine metabolism and function might be involved in the constellation of symptoms encompassing fatigue, anergia, reduced pleasure/tone, decreased motivation, and altered sleep and appetite [1, 2, 33, 34]. Consistent with this scenario, cytokines are responsible for the selective and time-dependent activation of specific enzymes, including indoleamine 2,3-dioxygenase (IDO) and GTP-cyclohydrolase 1 (GTP-CH1) that play a major role in serotoninergic, glutamatergic, and dopaminergic neurotransmission. Information on these enzymatic pathways and their role in monoamine biosynthesis and activity is fully discussed in this volume (see the chapters by Dantzer, Felger, and Fuchs in this book). Briefly, the sustained activation of brain IDO both at the periphery and in the brain results in an increased catabolism of tryptophan, the essential amino acid precursor of serotonin (5-HT), along the kynurenine pathway [1, 27, 31, 32]. This reaction can potentially reduce in turn tryptophan bioavailability for the synthesis of 5-HT although the extent to which this is the case in the real world remains to be demonstrated. More importantly, increased brain kynurenine levels can be further metabolized into neuroactive glutamatergic compounds, including 3-hydroxykynurenine and quinolinic acid, which are neurotoxic by stimulating NMDA receptors and promoting oxidative stress [35–37]. On the other hand,

kynurenine can also be metabolized into kynurenic acid that has neuroprotective properties to some extent. These apparently antagonistic pathways are compartmentalized in the brain, with microglia preferentially producing quinolinic acid and astrocytes kynurenic acid. Sustained immune activation therefore tips the scale in favor of neurotoxicity. In support of this notion, increased brain or cerebrospinal fluid (CSF) concentrations of kynurenine and its neurotoxic metabolites have been reported in several neurodegenerative and psychiatric disorders and they have been associated with extent of brain damage [35, 37–41], and with mood and cognitive impairments [37]. In addition, activation of the kynurenine pathway has been recently shown to affect human hippocampal neurogenesis [42–44].

Cytokine-induced activation of GTP-CH1, the rate limiting enzyme of GTP conversion ultimately leads to the production of neopterin by activated peripheral and brain immune cells. This is done at the expense of the formation of tetrahydrobiopterin (BH4), an essential co-factor of dopamine and serotonin biosynthesis [45–47]. Of note, BH4 is also a co-factor of nitric oxide synthase (NOS). By impairing the function of this enzyme, cytokines can indirectly contribute to increase the production of free radicals generating oxidative stress. This reaction in turn promotes oxidative reduction of BH4 itself, which is highly redox-sensitive, and by doing so decreases even more its availability for dopamine and serotonin syntheses.

The activation of IDO and GTP-CH1 and their respective related pathways by inflammatory processes may serve as key steps in the induction of inflammation-associated relevant neurobehavioral symptom constellations. More specifically, it appears highly possible that, given its role on serotonin and glutamate systems, IDO plays a major role in the development of mood and cognitive symptom domains whereas GTP-CH1, by modulating dopamine synthesis, could represent a pivot biological substrate of the neurovegetative and motivational symptoms associated with inflammation (for review see [33]). This scenario is currently supported by clinical and experimental evidence. At the clinical level, tryptophan levels and increased kynurenine/tryptophan ratio correlate with IFN-α-induced mood and cognitive symptoms but not with neurovegetative symptoms in medically ill patients undergoing immunotherapy [48]. In the same manner, decreases in blood tryptophan levels and increases in CSF quinolinic acid levels correlate with the intensity of depression scores in IFN-α treated patients, supporting further the involvement of kynurenine-related neurotoxic processes in the development of cytokine-induced depression [49]. These findings are consistent with recent data showing increased quinolinic acid levels in suicide attempters [50] and documenting a significant association between severe depression and increased microglial quinolinic acid in subregions of the anterior cingulate gyrus in post-mortem studies of depressed patients [39]. In accordance with these clinical data, preclinical findings in laboratory rodents demonstrate that activation of brain IDO is associated with cognitive and emotional alterations following immune activation [51–54] and that pharmacological or genetic inhibition of IDO activity, obtained by acting either directly on the enzyme or indirectly on its inducing cytokines (mainly IFN-γ and TNF-α), prevents inflammation-induced depressive-like behaviors,

anxiety-like behaviors, and/or cognitive impairment without impacting sickness behavior [27, 28, 31, 32, 51, 54, 55]. Supporting a role for the neurotoxic kynurenine metabolites in inducing behavioral alterations, NMDA receptor blockade abrogates cytokine-induced depressive-like behavior [56]. Consistent with the hypothesis that cytokine-induced activation of GTP-CH1 contributes to the development of neurovegetative and motivational symptoms, IFN-α-induced fatigue, decreased motivation, and anhedonia in IFN-α-treated patients and nonhuman primates correlate with significant alterations in BH4 [57] and dopamine neurotransmission [34]. In rats, systemic administration of IFN-α decreases brain levels of dopamine and BH4 through a mechanism involving NO, since this effect is reversed by treatment with an NOS inhibitor [58].

2.2 Vulnerability and Modulatory Factors

In addition to alterations in neurotransmitter metabolism, other non-exclusive mechanisms may contribute and/or facilitate the development of inflammation-induced mood/cognitive and neurovegetative/motivational symptoms. They involve the hypothalamo-pituitary-adrenal (HPA) axis, metabolic factors, and individual factors related to personality. Interestingly, recent data suggest that, depending of the involvement of these factors and their interaction with inflammatory processes, the presentation and expression of neuropsychiatric symptom dimensions may be different (Fig. 2).

The HPA Axis and Its Relation to Stress

Alterations in the HPA axis are a common feature in depression, and sensitization of this system, notably as a result of chronic stress or early life stress exposure, represents a potent risk factor for depression [59, 60]. Cytokines are capable of activating the HPA axis, leading ultimately to an increased production of glucocorticoids with strong anti-inflammatory actions. In patients treated with IFN-α, the first administration of the cytokine is responsible for a significant elevation in circulating levels of adrenocorticotropic hormone (ACTH) and cortisol [61]. Interestingly, this increase is threefold higher in patients who develop IFN-α-induced depression in comparison to patients who remain free of depression during treatment, and it correlates primarily with the development of the mood and cognitive features of depression. No significant association is found with the neurovegetative features of the disorder [61]. This result suggests that cytokine-induced HPA axis hyperactivity plays a distinctive role in the development of the mood and cognitive symptom dimensions related to inflammation. Supporting further the role of HPA axis alterations in modulating neuropsychiatric symptom presentation, multiple findings in depressed patients indicate that HPA axis hyperactivity is more apparent in depression with melancholic features. In depression with atypical features

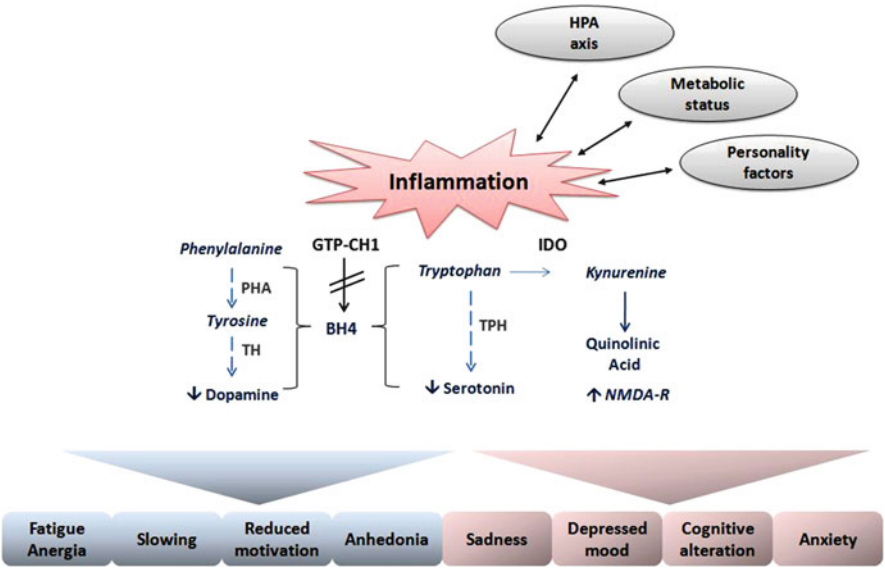

Fig. 2 Pathways of inflammation-induced neuropsychiatric symptom dimensions. Cytokines are responsible for the activation of the enzymes indoleamine 2,3-dioxygenase (IDO) and GTP-cyclohydrolase 1 (GTP-CH1) that play a major role in monoamine biosynthesis. Activation of IDO results in the increased catabolism of tryptophan, the essential amino acid precursor of serotonin (5-HT) along the kynurenine pathway. Kynurenine can be further metabolized to produce neuroactive glutamatergic compounds, including 3-hydroxykynurenine and quinolinic acid, which are neurotoxic by stimulating NMDA receptors and promoting oxidative stress. The activation of this pathway may play a role in the development of inflammation-related mood and cognitive symptoms. The activation of GTP-CH1 by inflammatory factors is done at the expense of the formation of tetrahydrobiopterin (BH4), an essential co-factor of dopamine (DA) and serotonin (5-HT) biosyntheses and nitric oxide synthase. In addition to its impact on 5-HT related functions and symptoms, the alterations of BH4 pathway may contribute to the development of neurovegetative symptoms that are more related to alterations in dopamine function, including fatigue, motor slowing, reduced motivation, and anhedonia. The presentation of inflammation-induced neuropsychiatric symptom dimensions is also modulated by the interaction of inflammatory processes with neuroendocrine (HPA) and metabolic factors, and with individual characteristics (personality factors)

(including mood reactivity, increased appetite/weight gain, hypersomnia/fatigue, and interpersonal rejection sensitivity) hyperactivation of the HPA axis is less obvious [62, 63].

Metabolic Abnormalities

Chronic low-grade inflammation is a fundamental characteristic of metabolic disorders, including obesity and the metabolic syndrome that are also associated with alterations in the HPA axis. Moreover, and consistent with the role of inflammation in the development of neuropsychiatric symptoms, those conditions are

associated with a greater prevalence of depressive, anxiety, and cognitive symptoms [64–66]. Depression is frequent in individuals afflicted with overweight or obesity, and several data indicate that atypical symptoms of depression, including the neurovegetative symptoms of increased appetite/weight gain, hypersomnia, and fatigue, are particularly frequent in those subjects [67–69]. Interestingly, substantial alterations in the activity of the brain reward system and dopamine function, similar to those described in patients with cytokine-induced depression [34, 70], have been also documented in different studies in obese subjects [71, 72]. These findings suggest that metabolic factors can contribute to the presentation of depressive symptom domains and thus modulate the expression of these symptoms when driven by inflammation. Consistent with this notion, hyperleptinemia is associated with major depression with atypical symptoms in both remitted and current depressed subjects, and this relationship is stronger in subjects having high adiposity levels [73]. In accordance with this data, metabolic alterations, including increased weight and body mass index (BMI), high triglycerides levels, and low high-density lipid cholesterol together with high levels of inflammation significantly discriminate depressed patients with atypical features from depressed subjects with melancholic depression [62]. Altogether, these findings support the hypothesis that inflammatory-metabolic interactions can differentially modulate the expression of depressive symptom dimensions.

Personality

Personality factors may interact with inflammatory processes to influence or exacerbate neuropsychiatric symptom presentation. In support of this, depressive symptoms in medically ill patients undergoing IFN-α therapy present differently depending on the type of population treated. In cancer patients, with no or only minor psychiatric antecedents, depressed mood, anhedonia, and fatigue symptoms were particularly frequent in the clinical presentation of IFN-α-induced depression, whereas irritability, anxiety, and mood reactivity/lability were more prevalent in IFN-α-treated chronic hepatitis C patients, who frequently have a personal history of drug abuse or risky behaviors [11, 74]. Supporting further the role of personality factors in these effects, neuroticism, pessimism, and reduced self-efficacy are associated with the intensity of fatigue and depressive symptoms in cancer patients treated with IFN-α [75, 76]. Interestingly, depressed patients with higher irritability have atypical features and exhibit higher novelty seeking, irritable and hyperthymic temperament scores when compared to patients with major depression and no irritability, who present with higher scores of harm avoidance [77].

3 Conclusion and Therapeutic Implications

The finding that cytokines are elevated in various neuropsychiatric disorders brings to the forefront the necessity of identifying the precise research domain criteria that inflammation is responsible for. Data gained with the model of inflammation-induced depression have allowed identifying distinct pathophysiological pathways involved in neurotransmitter metabolism with specific relevance for the development of symptom constellations encompassing, respectively, mood/cognitive symptoms and neurovegetative/motivational alterations. These symptom domains represent core features of various neuropsychiatric and neurodegenerative disorders that are also characterized by an activation of inflammatory processes. Interestingly, converging data indicate that the interaction of inflammatory processes (and their effect on neurotransmitter pathways) with relevant vulnerability or modulatory factors, including personality, metabolic, and neuroendocrine factors, may also contribute to influence the presentation of inflammation-driven neuropsychiatric symptoms. While further research is needed to precisely determine the specific mechanisms of action and sequence of events involved in the shaping of symptom dimensions related to inflammation and their generalization across conditions, these findings give to inflammation a central position in the RDoC strategic approach of mental health. They also suggest that treatments targeting inflammation and modulating the pathways and systems by which inflammatory processes selectively affect brain function and behavior may be of particular relevance for the treatment of specific neurobehavioral symptom domains. This strategy adheres to the principles that are required for the development of a precision medicine in psychiatry.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

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Inflammation-Associated Co-morbidity Between Depression and Cardiovascular Disease

Angelos Halaris

Abstract Morbidity and mortality of cardiovascular disease (CVD) is exceedingly high worldwide. Depressive illness is a serious psychiatric illness that afflicts a significant portion of the world population. Epidemiological studies have confirmed the high co-morbidity between these two disease entities. The co-morbidity is bidirectional and the mechanisms responsible for it are complex and multifaceted. In addition to genetic, biological systems, psychosocial, and behavioral factors that are involved include the central and autonomic nervous systems, the neuroendocrine, immune, and the vascular and hematologic systems. Specific pathophysiologic factors across these systems include homeostatic imbalance between the sympathetic and the parasympathetic systems with loss of heart rate variability (HRV) in depression, sympathoadrenal activation, hypothalamic–pituitary–adrenal (HPA) axis activation, immune system dysregulation resulting in a pro-inflammatory status, platelet activation, and endothelial dysfunction. These abnormalities have been demonstrated in most individuals diagnosed with major depressive disorder (MDD), bipolar disorder (BPD), and probably in other psychiatric disorders. A likely common instigator underlying the co-morbidity between cardiovascular pathology and depression is mental stress. Chronic stress shifts the homeostatic balance in the autonomic nervous system with sustained sympathetic overdrive and diminished vagal tone. Diminished vagal tone contributes to a pro-inflammatory status with associated sequelae. Stress hormones and certain pro-inflammatory substances released by macrophages and microglia upregulate the rate-limiting enzymes in the metabolic pathway of tryptophan (TRP). This enzymatic upregulation stimulates the kynurenine (KYN) pathway resulting in

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the formation of neurotoxic metabolites. Inflammation occurs in cardiac, cardiovascular, and cerebrovascular pathology independent of the presence or absence of depression. Inflammation is closely associated with endothelial dysfunction, a preamble to atherosclerosis and atherothrombosis. Endothelial dysfunction has been detected in depression and may prove to be a trait marker for this illness. Thus understanding vascular biology in conjunction with psychiatric co-morbidity will be of critical importance. Antidepressant drug therapy is of definite benefit to patients with medical and psychiatric co-morbidity and may reverse the pro-inflammatory status associated with depression. There is, however, an urgent need to develop novel pharmacotherapeutic approaches to benefit a much larger proportion of patients suffering from these disease entities.

Keywords Brain–immune interaction • Cardiovascular disease • C-reactive protein • Cytokines • Depression • Endothelial dysfunction • Heart rate variability • Inflammation • Kynurenine • Stress

Contents

1 Introduction

Cardiovascular disease (CVD) and depressive illness (DI) are two of the world's leading health problems. CVD is the leading cause of mortality worldwide and accounts for approximately 16.7 million deaths every year [1–3]. The most

common type of CVD is coronary artery disease (CAD) which is a leading cause of morbidity and mortality in the industrialized world and the leading cause of death and hospital admissions in the USA where CVD is responsible for one million deaths and more than six million hospital admissions annually [4]. DI afflicts an estimated 120 million people worldwide and is the leading cause of disability [5]. Incidence and prevalence rates vary somewhat depending on the part or the world the study was conducted and the methodology used. DI is a broad term comprising different subtypes the most common of which is Major Depressive Disorder (MDD). Bipolar Disorder (BPD) is of much lower prevalence than MDD but can be profoundly debilitating and has a much higher suicide rate. Depression associated with BPD presents major management challenges to the practitioner. MDD and BPD are chronic and recurring conditions with less than half of the patients achieving remission. The co-morbidity between DI and CVD is well known, high, and bidirectional. Factors contributing to the high co-morbidity between these disease entities are complex and multifactorial and not fully elucidated. A connection between mood disturbances and the immune system dates back to more than two decades ago [6–9] and continues to be actively studied. Therefore activation of inflammatory processes as a common link between DI, CVD, and cerebrovascular disease (CBVD) continues to be the focus of intense research endeavors.

Pro-inflammatory cytokines have been implicated in the pathogenesis of atherosclerosis and CVD [10–12]. Endothelial damage leads to the release of pro-inflammatory cytokines which induce a sequence of events leading ultimately to thrombus formation and vascular occlusion. Inflammation is a major contributing causal factor to DI and has been postulated to play a major role in endothelial damage of the cerebral vasculature. This has led to the designation of "vascular depression" as a pathophysiologically distinct variety that presents with many of the typical features of depression. Interestingly, elevations in inflammatory markers have been shown in depressed patients with or without CVD [13–15] and in cancer patients with depression [16]. One cytokine, IL-6, secreted in response to stress, is one of the most potent stimulators of the hypothalamic–pituitary–adrenal axis (HPA) axis, and induces the release of other pro-inflammatory cytokines. These and related observations have led Leonard to postulate that DI is a disease of inflammation in response to chronic psychological stress [17–20]. Indeed stress may be the common denominator in the etiopathology of DI, CVD, and CBVD in conjunction with genetic vulnerability. Since a sustained pro-inflammatory status has been clearly associated with widespread pathology, the assertion can be made that DI is a whole-body disease.

2 Epidemiology

2.1 Depressive Illness

DI is a serious medical illness comprising a multitude of cognitive, affective, and physical symptoms and characterized by recurrence and a chronic course. It is associated with higher rates of chronic physical disease, increased health care utilization, and impaired functioning. Suicidality is common, and can have a lethal outcome that may not be preventable. Rates of access to treatment remain low, the treatment received is often inadequate, and response rates are unacceptably low. Only one third of patients diagnosed with MDD or BPD achieve symptom remission, while a full one third fail to respond at all even after multiple antidepressant drug trials. It is estimated that 120 million people worldwide are afflicted with DI of one type or another. It is the leading cause of disability worldwide [21]. The lifetime prevalence of DI is as high as 16%, while the 12-month prevalence ranges from 3% in Japan to over 9% in the USA [5, 22, 23]. The incidence of DI has been steadily increasing over the past decades. Using the DALY, Disability Adjusted Life-Years, MDD was classed in 1990 as the fourth leading burden of disease worldwide for both sexes; by 2004, it advanced to the third place. According to the World Health Organization's estimate, DI will rank second to Heart Disease (HD) by the year 2020 [24] and will be the leading cause of disease burden by 2030 [5].

Of direct relevance is data presented in the National Health and Nutrition Examination Survey, 2009–2012 [25]. The survey examined depression and depressive symptom severity in the previous 2 weeks from a symptom-based questionnaire, by demographic characteristics, functioning difficulties, and recent contact with a mental health professional. Severity was categorized as severe, moderate, mild, or no depressive symptoms. During 2009–2012, about 7.6% of Americans aged 12 and over had depression (defined as having moderate or severe depressive symptoms in the past 2 weeks). Depression was more prevalent among females than males and among adults aged 40–59 than those of other age groups. Rates of any depressive symptoms were lower among non-Hispanic white persons than among Hispanic and non-Hispanic black persons. Once poverty was taken into account, however, the rates of depression did not differ significantly by race or Hispanic origin. Lastly, persons with mild depressive symptoms, as well as those with moderate or severe depressive symptoms, reported difficulties with work, home, and social activities related to their symptoms. Numerous studies have also shown that persons with depression have more functional limitations than those without depression.

2.2 Cardiovascular Disease

CVD is the leading cause of mortality worldwide and accounts for approximately 16.7 million deaths every year [4, 26, 27]. The most common types of CVD are CAD and CBVD. CAD is a leading cause of morbidity and mortality in the industrialized world [28] and it is the leading cause of death and hospital admissions in the USA. It has been estimated that CVD is responsible for close to one million deaths and more than six million hospital admissions with an annual cost to the US economy in excess of $350 billion [21]. Recent statistical data obtained in the USA reveal that about 610,000 people die of HD every year – that is, 1 in every 4 deaths [29]. HD remains the leading cause of death for both men and women. More than half of the deaths due to heart disease in 2009 were in men. CAD is the most common type of heart disease, killing over 370,000 people annually [29]. Every year about 735,000 Americans have a heart attack. Of these, 525,000 are a first heart attack and 210,000 happen in people who have already had a heart attack [30].

2.3 Co-morbidity Between DI and Cardiovascular Disease

Depression has long been associated with HD and death ("dying of a broken heart"). However, it has only been in the last 25 years that the scientific underpinnings supporting this common wisdom have been discovered. Since the early 1990s, studies have reported the prevalence of MDD to range between 17 and 27% in hospitalized CAD patients. It is now recognized that the co-morbidity between DI and CVD does not occur by chance and the mechanisms responsible for this relationship are complex and multifactorial. This remarkably high co-morbidity is believed to be bidirectional. Numerous studies published over the past two decades have confirmed this association. In a meta-analytic study conducted by Barth et al. [31], clinical depression was identified as a significant risk factor for mortality in patients with coronary heart disease (CHD). The Multiple Risk Factor Intervention Trial (MRFIT) of middle-aged men established an association between depressive symptoms and all-cause mortality with a higher risk of CVD related death and more specifically stroke mortality [32].

Clinical depression is the leading risk factor for CVD. Depressed patients have a two- to fourfold risk of developing CVD at some point in their lifetime [33–35] and a similarly higher risk of dying after a cardiac event [36, 37]. With regard to congestive heart failure (CHF), depressed patients are at higher risk, while patients with CHF who become depressed are likely to have worse outcomes than those without depression [38, 39]. Several studies have shown that the presence of depressive symptomatology predicts future coronary events for initially healthy individuals, and a poor prognosis for those who have documented CVD [40–42]. The Framingham Heart Study identified a direct relationship between

depressive symptomatology and all-cause mortality in a very large cohort of original and offspring cohort participants over a 6-year follow-up period [43]. The Baltimore Epidemiologic Catchment Area Follow-up Study [44] clearly pointed out that the diagnosis of depression is associated with an increased odds ratio (OR) for adverse outcomes, for example, an OR of 4.5 for MI and an OR of 2.7 for stroke. Lastly, in spite of limitations inherent in a meta-analytic study, the study by Nicholson et al. [45] established significant associations between depression and CHD and concluded that depression can be an etiologic or prognostic factor in CHD. Indeed, according to McCaffery et al. [46] depression and vascular disease may share certain vulnerability genes.

The high incidence of depression among patients suffering from ischemic heart disease has been unequivocally demonstrated in epidemiological studies [47–49]. Depression is unambiguously recognized as an independent risk factor for CVD mortality between 6 and 18 months following myocardial infarction (MI) [50, 51] and its presence is associated with increased CVD morbidity [37]. In patients with an acute MI, depression is a risk factor for recurrent nonfatal infarction and cardiac mortality independent of cardiac disease severity [52, 53]. Acute coronary syndrome (ACS) is both psychologically and physiologically stressful, and it is common to attribute depression observed following ACS to stress [155]. Depression observed following ACS is common and associated with an increased risk of mortality. Medically healthy individuals who suffer from depression are at significantly increased risk of developing heart attacks and strokes later in life [155].

3 Factors Accountable for the Co-morbidity

While there is little doubt that DI and CVD are epidemiologically linked, the precise mechanisms that interactively link these two disease entities elude us. Complex and multifaceted biological, psychological, socioeconomic, sociodemographic, and biobehavioral factors have been unequivocally shown to be significant contributors to the co-morbidity. Stress is a crucial factor underlying both conditions, but there is individual variation in stress susceptibility, responsivity, and resilience that should be taken into consideration. Genetic and epigenetic factors exert powerful influences on shaping the individual's coping skills with stressful events, and with physical and mental illness in particular as chronic conditions. Genetic polymorphisms exert critical effects in conferring resistance or susceptibility to illness. Environmental factors acting via epigenetic mechanisms are only now beginning to be understood. The precise pathophysiological processes and biobehavioral factors that explain the mechanistic associations between the two entities are critical determinants in our ability to develop specifically targeted pharmacotherapeutic, psychotherapeutic, and preventive interventions.

The mechanistic pathways accountable for the co-morbidity between DI and CVD are complex and involve multiple systems. Indeed DI should be viewed as a "whole body disease" rather than a psychological abnormality involving mood dysregulation. Once clinical depression is present, a host of physiological changes occur, including ANS and immune system activation, neuroendocrine changes, rhythm disturbances, oxidative stress, platelet hypercoagulability, all of which exert a negative impact on cardiovascular health. The majority of studies seeking to elucidate the biological underpinnings of this co-morbidity have focused on a single or a handful of parameters making meta-analytic comparisons across studies difficult. Additionally, biobehavioral mechanisms play a critical role in defining the source of the high co-morbidity. For instance, life style factors, such as alcohol consumption and smoking, physical inactivity, and medication nonadherence, partly due to side effects associated with antidepressant medications, all play contributory roles to the relevant biological factors. Last, but not least, traditional cardiovascular risk factors, such as hypertension, diabetes, dyslipidemia, obesity, and substance abuse, especially nicotine dependence, play variable roles in defining co-morbidity and must always be precisely assessed in such studies. In spite of these barriers, however, significant findings and promising leads already exist and they can guide future research in this field. This review article focuses on the contribution of inflammation as critical factor underlying both disease entities and accountable for the established bidirectionality. Brain–immune interaction will also be mentioned as it relates specifically to aberrant serotonergic transmission believed to be responsible, at least in part, for some aspects of the profile of mood and related symptoms associated with depression.

3.1 Role of Stress in Inflammation and Depression

Mental stress can exert profound pathophysiological changes in the central and peripheral nervous systems, the immune, endocrine, and vascular systems with multiple organ involvement. Mental stress can be of many varieties with differences in intensity and duration, and the perception of mental stress is subject to high individual variability and vulnerability. Indeed genetically determined stress vulnerability is a key factor in determining what the precise consequences of stress will be in a given individual. This is based, at least in part, on genetically determined and epigenetically modified stress susceptibility and stress resilience. Any illness, physical or mental, is of itself stressful to the individual. Chronic and especially inescapable stress, such as stress associated with chronic illness – physical or mental – low socioeconomic status, dysfunctional relationships, caregiver status, ultimately leads to pervasive mental status changes, chronic low-grade inflammatory reaction, and pathological alterations to the function and structure of the cardiovascular and cerebrovascular system. Additionally, stress hormones and certain pro-inflammatory compounds upregulate enzymatic processes that lead to neurotransmitter deficiencies critical for mood regulation. Taken together, the

confluence of these factors will ultimately lead to irreversible tissue and organ damage. As Selye [54] had postulated, when the demands placed on the organism eventually exceed the available energy and adaptation, the system fails. The complexity of the interactions between stress, and medical and psychiatric illnesses remains a topic of intense scientific endeavor.

In addition to activating the HPA, stress activates the sympathetic branch (SNS) of the ANS with a concomitant reduction in vagal tone. Diminution in parasympathetic tone affects the body's immune response. This effect occurs, at least in part, through the cholinergic anti-inflammatory pathway (CAIP), as described by Tracey [55]. The imbalance in ANS function, especially if prolonged, as is invariably the case with psychiatric disorders, has profound effects on cardiovascular physiology and the immune system. These pathophysiological changes contribute significantly to the co-morbidity between CVD and psychiatric disorders. Thus, to quote Thayer and Lane [56], autonomic imbalance and decreased parasympathetic activity in particular may be the final common pathway to numerous diseases and conditions associated with increased morbidity and mortality.

3.2 Autonomic Nervous System Dysfunction

The stress-related disruption in ANS homeostasis with a sustained shift in the balance of the sympathetic and parasympathetic branches of the ANS is a critical underlying mechanism in as much as depression is the stress-related psychiatric disorder par excellence. The degree and chronicity of disruption in ANS homeostasis appears to be the tipping point determining the extent of diminution in parasympathetic tone. Diminution in vagal tone likely leads to disinhibition of the body's inflammatory response mediated, in part, by the CAIP [55, 57] by efferent vagal fibers originating in the dorsal motor nucleus. These efferent fibers can modulate the release of inflammatory mediators, such as Tumor Necrosis Factor alpha (TNFα), from macrophages thereby preventing over-activation of the inflammatory process without inducing immunosuppression [58]. In a more recent publication, Pavlov and Tracey [59] discuss in greater detail the brain–immune interaction and provide supportive evidence that the issue is more complex than was originally conceived by these authors.

Sympathoadrenal (SA) hyperactivity in depression leads to increases in plasma catecholamines, vasoconstriction, elevated heart rate, and platelet activation. The Heart and Soul Study established that in patients with CAD depressive symptoms are associated with elevated levels of norepinephrine [60]. These changes, singly and additively, exert adverse effects on the cardiovascular system. Sustained catecholaminergic action on the heart, blood vessels, and platelets leads to changes in hemodynamic factors with increased shear stress. The concomitant decrease in parasympathetic tone may predispose to ventricular arrhythmias and possibly explain the excessive cardiovascular mortality found in patients with CVD and comorbid depression.

3.3 Heart Rate Variability

Heart Rate Variability (HRV) is a recognized measure reflecting fluctuations in ANS activity and is an index of a healthy heart and good cardiac function with ability to adapt to external and internal stressful demands. It is considered to be an independent predictor of death. Diminished high frequency HRV reflects decreased parasympathetic tone and has been observed at least in some depressed patients [61]. HRV can also be significantly decreased in severe CAD or heart failure. The risk of sudden death after acute MI is considerably higher with decreased HRV. Indeed HRV is a post-MI prognostic factor (along with age, left ventricular ejection fraction, and frequency of arrhythmia). It has been reported that in patients with CAD, diminished HRV is far more common in depressed rather than in matched nondepressed patients. In a large cohort study (NESDA), Licht and coworkers demonstrated that depressed patients, when compared to healthy control subjects, have significantly both lower total HRV and lower HRV in the respiratory frequency range (RSA), which reflects diminished cardiac vagal control [62]. They concluded that their findings are compatible with theoretical models, notably the Polyvagal Theory coined by Porges [63]. These models have linked the parasympathetic system to the etiopathology of DI based on the premise that low vagal tone is associated with reduced social engagement and an impaired response to environmental stimuli and challenges, as described by Porges [64, 65]. However, in a later publication Licht et al. [66] make a strong case that reduced HRV in depressed individuals may be a concomitant effect of antidepressant drugs and not only tricyclics but SSRIs and noradrenergic antidepressants. Clearly this issue requires more research to clarify the potential confounding effect of antidepressant drug treatment on HRV in spite of their beneficial effects.

A sustained low-grade inflammation in depression leads to a diversion of tryptophan (TRP) metabolism via stimulation of indoleamine 2,3-dioxygenase (IDO), a key enzyme responsible for the kynurenine (KYN) pathway [67]. As a result of this enzymatic activation, a metabolic product, kynurenic acid, antagonizes nicotinic cholinergic transmission. The complex interplay between the brain, the ANS, and the immune system ostensibly results in varying degrees of ANS disruption resulting in greater or lesser degrees of parasympathetic tone diminution. If parasympathetic tone can be maintained at healthy control levels, reflecting a lower or absent degree of inflammatory response and therefore lesser activation of the KYN pathway, antidepressant drug action may proceed unhindered ultimately leading to remission.

4 Bidirectionality of Inflammation in Cardiovascular Disease and Depression

4.1 Inflammation and Depression

The Cytokine Theory of Depression was originally formulated by Smith [68] as "The Macrophage Theory of Depression" and later expanded by Ur et al. [69]. The theory postulated that psychological stress, probably in conjunction with genetic factors, increases cytokine production and leads to depressive symptoms when specific neurobiological systems are affected, such as the HPA axis and serotonergic transmission. High circulating levels of pro-inflammatory cytokines may thus be central to the pathophysiology not only of MDD and BPD but also likely Atypical Depression and Posttraumatic Stress Disorder. Cytokines may firstly participate in the etiology of DI, and later in the high incidence of CAD observed amongst depressed patients [70].

The pro-inflammatory status associated with MDD has been adequately described in the literature ([18, 71–80]; for review see [81]). These reports demonstrate the increase of pro-inflammatory cytokines, notably, IL-2, IL-6, soluble IL-6 receptor, TNFα and IFN-γ, and the decrease in anti-inflammatory cytokines, such as IL-4 and IL-10. Studies of injected cytokines have shown that pro-inflammatory cytokines, by themselves, are dysphoric. They are capable of inducing a syndrome known as "sickness behavior" that simulates many of the classical symptoms of depression [6, 82, 83]. But while numerous previous reports have shown that pro-inflammatory cytokines are endogenously over-expressed in depression and other stress-induced disorders [84], not all reports agree. Indeed some reports have also shown below normal blood levels of pro-inflammatory cytokines stimulating further research into the possible factors accountable for discrepant findings. For a more detailed discussion, the reader is referred to critical reviews on this issue [8, 85–87].

Although the degree of cytokine elevations in DI is moderate in comparison to elevations during physical injury and/or infection [88], by being chronically over-expressed in DI cytokines can have long-term adverse consequences on the cardiovascular system [89]. Indeed pro-inflammatory cytokines have been shown to be causally linked to plaque formation, and CAD [90]. Therefore, overproduction of pro-inflammatory cytokines in DI especially over prolonged periods of time and in patients who fail to respond to antidepressant interventions may be one common link between DI and CAD [91–93].

Two relevant questions need to be addressed: (a) does a sustained pro-inflammatory status hinder the effectiveness of antidepressant drug treatments, and (b) does antidepressant therapy normalize elevated levels of pro-inflammatory mediators. With respect to the first question, we and others have postulated that the chronic pro-inflammatory status found in depression may delay, diminish, and even thwart antidepressant response. Furthermore, a persistent pro-inflammatory status is a likely contributor to the increased risk of depressed patients to develop potentially

life-threatening complications, such as CVD and CBVD, and even dementia. It has therefore been hypothesized that controlling inflammation without inducing immunosuppression might improve antidepressant treatment outcomes. A few small clinical studies already exist pointing to the add-on benefit of an anti-inflammatory compound, such as the COX-2 inhibitor, celecoxib, in combination with a standard antidepressant drug [94]. However, these have been small studies and, except for one, did not use a placebo-controlled double blind design. We have published our own preliminary study using escitalopram and celecoxib add-on in treatment resistant bipolar depression and the findings have confirmed the hypothesized advantage of combination therapy [97].

With respect to the second question, a number of reports indicate positive effects of antidepressant drug therapy in normalizing abnormally elevated levels of pro-inflammatory cytokines [75, 98–101]. Such a reduction can only be beneficial as has been clearly described in heart failure patients by Mayer et al. [102]. However, not all reports are consistent in this regard. Differences in study design, mainly the length of treatment and the type of antidepressant agent utilized, appear to be crucial variables that must be controlled in future studies. Additionally, the time course of cytokine normalization may not precisely coincide with the time frame of clinical antidepressant response. The article by Janssen et al. [103] offers a comprehensive review of cytokine involvement in antidepressant treatment response. Clarification of the temporal associations between mood improvement and cytokine normalization should be an important aspect of future studies [104].

4.2 Inflammation and Cardiovascular Disease

Inflammation plays a major role in the development of atherosclerosis and atherothrombosis that are associated with high morbidity and mortality [105]. Immune-competent cells are involved both in the early stages of atherosclerosis and the subsequent events leading to plaque formation and rupture and the ensuing ACSs [106]. Circulating compounds identified as markers of inflammation and atherosclerosis include acute-phase proteins, C-reactive protein (CRP), fibrinogen, immunoglobulins, adhesion molecules, and cytokines. Most of these biomarkers are abnormally regulated in patients with depressive syndromes without any evidence of other inflammatory processes, cardiovascular or immune system pathology. Fibrinogen, CRP and from within the cytokine group TNFα, interleukin-1 (IL-1), and interleukin-6 (IL-6) have been discussed extensively in the cardiovascular literature. Indeed Frasure-Smith et al. [107] have made a case that CRP and depression have prognostic value in predicting adverse cardiac events. The literature is replete with findings of significant and sustained elevations in plasma levels of *hs*CRP in depressed subjects that may or may not normalize even after symptom remission, indicating persisting CVD vulnerability even after full recovery from a depressive episode.

It is widely recognized that inflammatory processes in the atherosclerotic artery may lead to increased blood levels of pro-inflammatory cytokines and other acute-phase reactants. Blood levels of IL-6 and CRP are elevated in patients with MI or unstable angina, as are other inflammatory biomarkers, such as fibrinogen, IL-7, IL-8, and soluble CD40 ligand. These findings point to inflammation in the coronary arteries. Collectively, these circulating biomarkers indicate the presence of an ACS and may reflect the clinical course of the condition [108].

Heart failure (HF) is a serious syndrome that at best interferes with normal functioning and significantly impairs the capacity of an individual to lead a normal life. At worse, HF can have a fatal outcome with heart transplantation being a lifesaving alternative. Although several models have been proposed to understand the pathophysiology of this syndrome, none have proven to be critical for the prevention and management of this condition. To quote from an earlier publication by Seta et al., "heart failure progresses, at least in part, as a result of the toxic effects exerted by endogenous cytokine cascades on the heart and the peripheral circulation" [109]. That an inflammatory cascade is responsible, at least in part, for the progression of HF is supported by animal and clinical data [110]. With inflammatory processes now being better understood, improved and effective treatment approaches are more likely to become available.

4.3 C-Reactive Protein

CRP is an annular, pentameric protein synthesized by the liver in response to factors released by macrophages and adipocytes. It is an acute-phase protein and is upregulated by pro-inflammatory cytokines, such as IL-6, that are activated early in the inflammatory process. CRP has therefore been historically recognized as a nonspecific inflammation marker. Commonly studied within the context of vascular medicine, an elevated CRP is a risk factor for atherosclerosis and cardiovascular disease [111–113]. The association between CRP and disease, however, may extend beyond the field of cardiology. Recently, CRP has become the focus of psychiatric research, specifically as it relates to MDD and a number of studies have sought to characterize the exact relationship between CRP and MDD. Patients with MDD have been shown to exhibit significantly higher levels of CRP when compared to healthy control subjects [114]. This finding extends to the general population as demonstrated by Wium-Andersen et al. [115] who demonstrated an association between CRP levels and psychological distress in 73,131 men and women representative of the Copenhagen population. After adjusting for confounding variables such as age, sex, smoking, physical activity, and chronic disease, they noted that elevated levels of CRP correlated to an increased risk for psychological distress and depression. The link between CRP and MDD raises questions about potential cardiovascular risks for MDD patients and pharmacological interventions aimed at resetting immune activation as part of a comprehensive treatment strategy for DI.

A meta-analysis performed by Howren et al. [116] examined the inflammatory markers CRP, IL-1, and IL-6, in conjunction with depression. This study is particularly noteworthy because it is one of the largest meta-analyses examining CRP and depression and has gained greater generalizability than individual studies. This meta-analysis compared the statistical significance when you do, and do not, account for a patient's Body Mass Index (BMI). The study revealed that while there is still a relationship between high CRP and MDD after adjusting for BMI, when no adjustment is made for BMI the relationship is more notable. This suggests that BMI influences the association between CRP and MDD.

While many hypotheses have been proposed regarding the link between elevated CRP and depression, it appears a variety of covariates influence this relationship. From BMI and general physical health, to depression type and recurrence or chronicity, the correlation between CRP and MDD is likely multifactorial and bidirectional. This means that CRP, upregulated during immune system activation, has the potential to induce depressive symptomatology. One possible mechanism may involve the upregulation of indoleamine 2,3-dioxygenase (IDO) which diverts TRP to the kynurenine pathway (KP) thereby possibly diminishing serotonin synthesis (Myint et al. 2003). However, it appears that the production of neurotoxic metabolites is a more likely mediator and this possibility is discussed in greater detail in the chap. 6. Regardless of the numerous factors impacting CRP and MDD, the question remains as to why CRP, a reflection of inflammation, is related to MDD in the first place.

A prospective study conducted by Copeland et al. [117] concluded that an early elevation in CRP did not predict later depression, but rather that depression was associated with later high CRP levels. The study also noted that CRP levels increase in relation to the number of prior depressive episodes a person has had. This suggests that an aspect of MDD itself may be mediating the elevation in CRP levels that are found in depressed individuals. It also points to a unidirectional relationship of depression being associated with later elevation in CRP.

This presumptive mechanism linking CRP and depression may point toward a unidirectional relationship, namely, that the presence of depression leads to elevated CRP. This is certainly plausible. When the body is under physical assault, be it from a virus or bacteria, a stress response is triggered. Such a response results in the upregulation of pro-inflammatory cytokines, with CRP being representative of this inflammatory response. Depression can be viewed as a form of mental assault or psychological invasion posing a serious threat to the homeostatic balance of the organism. In this context, it has been proposed that organisms respond similarly to the stress associated with or caused by depression as they do to external stressors. Frank et al. [118] showed that in laboratory animals, psychological stressors, such as restraint and isolation, result in increased release of pro-inflammatory cytokines. In their paper, they discuss microglia serving as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. This issue is cogently discussed by Raison and Miller [119] suggesting that the mediating factor behind MDD and increased inflammation is the body's perceived level of stress.

A comprehensive discussion of CRP and depression far exceeds the scope of this review. However, it is still an unresolved question whether the treatment with antidepressant agents or mood stabilizers brings about a normalization of elevated blood levels of CRP. For a discussion of this issue, the reader is referred to a previous publication [104].

Lastly and before concluding this very brief overview on CRP, inflammation, DI, and CVD, it should be mentioned that CRP exists in at least two conformationally distinct forms, i.e., native pentameric CRP (pCRP) and modified/monomeric CRP (mCRP). It is believed that pCRP, what is commonly measured in routine laboratory assays, possesses both pro- and anti-inflammatory actions in a situation dependent manner. By contrast, mCRP exerts potent pro-inflammatory actions on endothelial cells, endothelial progenitor cells, leukocytes, and platelets, and thus may amplify inflammation. At this point, there is no established method that distinguishes these two forms of CRP in a routine laboratory measurement [120]. To my knowledge, it is not known what percentage, if any, of mCRP is contained in the routine measurement of *hs* CRP.

4.4 Endothelial Dysfunction

Endothelial dysfunction is a vascular phenotype predisposing to atheromatosis and arteriosclerosis and can thus serve as a predictor of cardiovascular events [121]. Dysfunction of the endothelium typically leads to immunological alterations, including activation, adhesion, and aggregation of platelets to areas of damage. Attachment of monocytes and lymphocytes to endothelial cells is mediated by cellular adhesion molecules comprising selectins and immunoglobulins. As noted earlier, the role inflammation plays in the pathogenesis of CVD is now widely accepted. Low-grade chronic inflammation is predictive of MI and ischemic stroke. Endothelial dysfunction is a "critical intermediate phenotype" in the relationship between low-grade inflammation and CVD [122].

Endothelial dysfunction can be viewed as an "intermediate phenotype" in DI based on the presence of a low-grade chronic inflammation in many depressed patients exemplified by mild to moderate elevations in circulating inflammation biomarkers. Depression may act as a chronic stressor that contributes to endothelial dysfunction through abnormalities in cellular adhesion, migration, and proliferation and platelet hypercoagulability [123–125]. Indeed depression has been associated with higher levels of intercellular adhesion molecule-1, p-selectin, and monocyte chemoattractant protein-1 (MCP-1) [78, 126]. Some investigators have postulated that endothelial dysfunction, viewed as a biomarker of arterial atheromatosis [127, 128], may prove to be a trait marker for depression [129]. In this context, impaired flow-mediated dilatation of the brachial artery has been described in MDD and BPD patients [130]. The study by Do et al. [131] focused on hopelessness as a frequent symptom of depression, which can escalate to suicidality, in association with markers of endothelial dysfunction. They concluded that negative

psychosocial traits may influence cardiovascular outcomes partially through their impact on the early stages of atherosclerosis, and specific psychosocial traits, such as hopelessness, may play a more direct role in this process than overall depressive symptoms. In summary, endothelial dysfunction is a crucial factor in the complex relationship between depression, low-grade chronic inflammation, and CVD.

5 The Tryptophan/Kynurenine Pathway: Implications for Serotonergic Transmission

A sustained pro-inflammatory status was initially thought to lead to deficient availability of serotonin in brain and periphery [132, 133]. Since CVD is accompanied by elevations in peripheral measures of immune system activation, it would be reasonable to attribute depression associated with CVD to deficient serotonergic transmission. A similar consideration would therefore apply to DI predisposing to CVD by virtue of a chronic pro-inflammatory state, along of course with a host of other factors that were discussed above. More recent evidence, however, has questioned the presumed effect of inflammation on brain TRP levels that remain stable and actually even increase during systemic inflammation, in contrast to the peripheral TRP levels. In their study of CSF concentrations of brain TRP and KYNs during immune stimulation with IFN-α, Raison et al. [134] determined that IFN-α had no effect on CSF TRP concentrations despite significant decreases in peripheral blood TRP.

What mechanisms might therefore be accountable for deficient serotonergic transmission that has been linked to depression? Certain pro-inflammatory cytokines, particularly interferon-γ, IL-1, IL-6, TNFα, and the acute-phase protein CRP, have been shown to induce the enzyme IDO. IDO is the rate-limiting enzyme of the KP which metabolizes TRP into KYN. KYN is further metabolized into several neurotoxins, notably 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), and quinolinic acid (QUIN). In a competing branch of the KP, KYN is metabolized into kynurenic acid (KA), which blocks the neurotoxic effects of QUIN. Another enzyme, tryptophan 2,3-dioxygenase (TDO) is activated by stress hormones, notably cortisol, and also contributes to the activation of the KP.

Therefore, the generation of neurotoxic KYN metabolites as a consequence of immune activation of IDO and kynurenine monooxygenase (KMO), the key enzyme for the generation of 3-HK and QUIN, must be given due consideration in further understanding inflammation-induced depression. Indeed we have shown a beneficial effect of escitalopram in MDD patients treated with this SSRI for 12 weeks and showing diminution in 3-HK and QUIN in the blood of these patients leading us to postulate a neuroprotective effect of this SSRI [135].

6 Inflammation and the Serotonin Transporter

Additional mechanisms play key roles in mediating the association between DI and inflammation. Activation of the serotonin transporter (SERT) by cytokines has been known for some time. In an earlier study, Zhu et al. [136] described IL-1R- and p38 MAPK-dependent regulation of SERT as one of the mechanisms by which environmentally driven immune system activation can trigger despair-like behavior in an animal model. These investigators proposed that future analysis of this pathway could be important in identifying risk factors in neuropsychiatric disorders. In a recently published animal study, the same group of investigators [137] reported that peripheral activation of the innate immune system with LPS led to a rapid stimulation of central nervous system SERT activity, accompanied by an acceleration of 5-HT clearance rate and alterations in SERT-dependent behaviors. An upregulation of SERT by certain pro-inflammatory cytokines could lead to decreased postsynaptic neurotransmitter availability. On the other hand, SPECT imaging studies showed that SERT availability was significantly lower in the midbrain and caudate of patients with BPD compared with healthy control subjects, but not in the thalamus and putamen. IL-10 was significantly higher, whereas TNFα was not different in euthymic patients with BPD compared with healthy controls. There was a significant association of SERT availability and IL-10 in the thalamus, but not in the midbrain, caudate, or putamen. The authors interpreted their results as demonstrating an interaction of SERT availability and IL-10 in euthymic BPD subjects [138]. In their MDD study, Amsterdam and colleagues found overall lower binding of ^{123}I-ADAM binding to SERT with a significant increase after cognitive behavioral therapy in their drug free depressed subjects. These investigators concluded that the low SERT binding seen during depression may reflect lower brain serotonin levels via a compensatory SERT downregulation. Thus, low SERT binding in depression may reflect an overall low serotonin function in depression that "normalizes" during response to treatment [139]. Taken together, the data from animal studies do not agree with the reports from human SERT imaging. Larger studies are required with medication free patients and inclusion of genotyping to ascertain the underlying SERT polymorphisms in the participants.

7 The Glutamatergic Theory of Depression

Glutamate and glutamatergic transmission have been gaining increasing importance in understanding the complex etiopathology of affective disorders since the discovery that ketamine administration can produce profound and rapid response in treatment resistant depressed patients [140, 141]. Ketamine acts as an antagonist of the glutamate N-methyl-D-aspartate receptor (NMDA) [142]. What are the possible mechanisms that link inflammation and glutamatergic transmission to affective

disorders? As mentioned above, IDO stimulation by cytokines activates the KP and leads to increased formation of QUIN by activated microglia and macrophages in the brain [143]. QUIN binds to the NMDA receptor but also stimulates the release and inhibits the reuptake of glutamate by astrocytes [144]. The ratio of the neuroprotective metabolite KA to QUIN is lower in depressed patients and correlates negatively with anhedonia [145] whereas in another study QUIN correlated positively with IL-6 in suicide attempters [146].

Several teams of investigators have shown that pro-inflammatory cytokines decrease the expression of glutamate transporters on astrocytes and increase astrocytic glutamate release [147, 148]. Astrocytic release of glutamate binds preferentially to extrasynaptic NMDA receptors which decrease neurotrophins and increase excitotoxicity [149]. To quote A. Miller, "the kynurenine pathway in general and quinolinic acid in particular may represent another point of convergence of the impact of inflammation and glutamate pathways on the brain and behavior" [150]. This brief review would be incomplete without mentioning the elegant work by Haroon and coinvestigators. Utilizing magnetic resonance spectroscopy, this team has shown that administration of the inflammatory cytokine IFN-α increases the glutamate to creatine ratio in the left basal ganglia and dorsal anterior cingulate cortex both of which have been implicated as targets of peripherally administered inflammatory stimuli. Increased glutamate in these brain regions correlated with depressive symptoms including anhedonia and fatigue [151–153]. These issues are discussed in greater detail by chap. 40.

8 Other Mechanisms

Lastly, activation of GTP-cyclohydrolase1 decreases the bioavailability of tetrahydrobiopterin, an important cofactor for all mono-oxygenases (including TRP hydroxylase but also phenylalanine and tyrosine hydroxylases). This mechanism is discussed by chap. 14.

In their comprehensive review article, Haase and Brown [154] discuss the complex interplay between pro-inflammatory cytokines, SERT, and neurotrophins, specifically brain-derived neurotrophic factor (BDNF). As a growth factor expressed predominantly in hippocampal neurons, it is presumed to exert a neuroprotective function. If BDNF release declines in conjunction with a pro-inflammatory state, neuronal dysfunction with emergence of depressive symptoms ensues.

9 Treatment Issues

With respect to access to treatment, the data from the National Health and Nutrition Examination Survey, 2009–2012 present some stunning findings [25]. Just over one-third (35.3%) of persons with severe depressive symptoms reported having seen a mental health professional in the past year, while less than 20% of all Americans with moderate depressive symptoms had been seen by a mental health professional in the past year. Finally 13% of persons with mild symptoms had seen a mental health professional in the past year. On a somewhat positive side, these figures indicate that the rates of seeing a mental health professional increased as severity of depressive symptoms increased in all race and Hispanic origin groups. With respect to treatment modality and associated effectiveness, the survey indicates that the most effective treatment for depression, especially for severe depression, is a combination of medication and psychotherapy. As Glassman et al. pointed out several years ago, depression aggravates the course of multiple cardiovascular conditions and has regularly been shown to lower adherence to prescribed medication and secondary prevention measures [155]. Therefore the need for multipronged treatment modalities that include proven efficacious and safe pharmacologic and nonpharmacologic interventions has never been more urgent. To date, few randomized controlled trials have evaluated the efficacy of treatments for MDD in patients with CVD and flowing MI or CHF. Additionally, innovative studies should be undertaken to focus on the inflammation aspect of both disease entities in designing innovative preventive, therapeutic, and rehabilitative interventions.

10 Concluding Remarks

This review article has attempted to summarize compelling evidence gathered from multiple epidemiological, clinical, and basic studies that DI and CVD are intricately associated with each other. The co-morbidity is firmly established but the relationship between these two serious disease entities is complex and multifaceted. Based on the available literature, inflammation has emerged as a dominant theme and a major mechanism contributing to the co-morbidity. An active inflammatory process is present during the active stages of either illness, likely precedes the emergence of debilitating symptoms, and possibly extends beyond mood normalization and symptom remission. To address these complex questions, integrated multidisciplinary teams of clinician investigators with diverse expertise in basic laboratory, clinical, and cardiodiagnostic settings must coalesce. Such integrated teams are unfortunately rare. However, it is only through the cohesive interaction of such multidisciplinary teams that we will ultimately succeed in unraveling the complex relationships between mental stress, inflammation, immune responses and depression, CVD and CBVD. Additionally, formalized training of psychiatrists

and cardiologists must be designed, possibly as a subspecialty, as I and others have proposed [156]. In addition to broadening and intensifying basic, clinical, and translational research efforts, innovative pharmacotherapeutic agents must be developed based on discoveries of alternate pathways involving interactively the immune and neurotransmitter systems. The efficacy of serotonin reuptake inhibitors in relieving depression and in reversing at least some of the pathogenetic factors common in both disease entities, although established and widely available, is a far cry from achieving symptom remission in the majority of such patients. Additionally, preventive measures and lifestyle changes that will hopefully reduce the current disease burden must be designed and implemented on a population-wide basis. The ultimate goal is to serve more effectively these patient populations by arriving at an early and accurate diagnosis, and utilizing innovative treatment approaches and preventive strategies.

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Immune-to-Brain Communication Pathways in Inflammation-Associated Sickness and Depression

Charlotte D'Mello and Mark G. Swain

Abstract A growing body of evidence now highlights a key role for inflammation in mediating sickness behaviors and depression. Systemic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and chronic liver disease have high comorbidity with depression. How the periphery communicates with the brain to mediate changes in neurotransmission and thereby behavior is not completely understood. Traditional routes of communication between the periphery and the brain involve neural and humoral pathways with TNFα, IL-1β, and IL-6 being the three main cytokines that have primarily been implicated in mediating signaling via these pathways. However, in recent years communication via peripheral immune-cell-to-brain and the gut-microbiota-to-brain routes have received increasing attention for their ability to modulate brain function. In this chapter we discuss periphery-to-brain communication pathways and their potential role in mediating inflammation-associated sickness behaviors and depression.

Keywords Cytokines • Depression • Gut microbiome • Microglia • Sickness behavior

Contents

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Animal models have highlighted a key role for three main cytokines, namely TNFα, IL-1β, and IL-6, in mediating communication between the periphery and the brain during systemic inflammation. As a result of this communication, systemic inflammation is often accompanied by changes in CNS function that lead to behavioral alterations including fatigue, social withdrawal, cognitive dysfunction, and loss of motivation (collectively termed sickness behaviors) [1, 2]. Administration of the Gram negative cell wall component lipopolysaccharide (LPS; a potent inducer of TNFα, IL-1β, and IL-6) or the three respective cytokines to rodents or healthy subjects results in the development of sickness behaviors and depressed mood [3, 4]. Furthermore, blockade of systemic cytokine (e.g., TNFα) signaling improves fatigue-like behaviors in animal models, including in mice with liver inflammation induced by bile duct ligation (BDL) [5]. Clinical observations in patients with systemic inflammatory diseases associated with high incidence of sickness behaviors and altered mood also parallel findings observed in animal models. In patients with a range of peripheral organ inflammatory diseases including chronic liver disease [6], psoriasis [7], inflammatory bowel disease (IBD) [8], diabetes [9], or rheumatoid arthritis [10], circulating levels of cytokines such as TNFα or IL-6 are elevated. Anti-TNFα therapy can lead to improvements in patients' sense of well-being and associated health related quality of life (HRQOL), well before overt changes in tissue inflammation [11]. Similarly, in patients with rheumatoid arthritis, either anti-TNFα or anti-IL-6 therapy often results in striking and rapid improvements in HRQOL, well before noticeable changes in joint inflammation [12]. Consistent with this finding, functional MRI (fMRI) has shown that anti-TNFα therapy in patients with rheumatoid arthritis rapidly (within 24 h) alters brain activation in response to a painful peripheral stimulus [13]. The potential importance of cytokine signaling to the brain in altering mood is further supported by a recent pilot study in patients with treatment-resistant depression in whom anti-TNFα therapy improved depressive symptoms; specifically in the subgroup of patients with high baseline inflammatory biomarkers [14]. Cytokine polymorphisms are also known to influence disease susceptibility and severity in systemic inflammatory diseases. For instance, genes involved in TNFα signaling and its key intracellular mediator NF-kB have been implicated in the pathogenesis of many systemic inflammatory

diseases [15]. In addition, TNFα polymorphisms have also been linked with fatigue and mood disorders [16, 17].

Communication between the periphery and the brain has been postulated to occur via four main pathways: (1) neural pathways, (2) signaling via cerebral endothelial cells (CECs), (3) signaling via circumventricular organs (CVOs), and (4) peripheral immune-cell-to-brain signaling (Fig. 1).

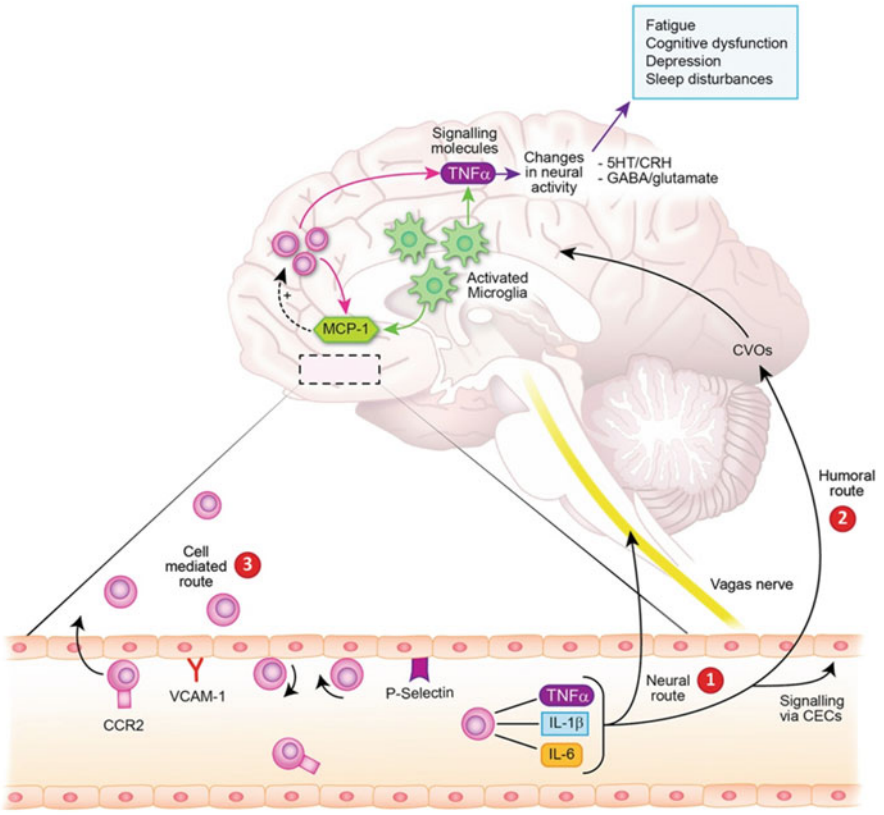

Fig. 1 Periphery-to-brain communication pathways (*1*) Neural route: visceral organs are innervated by vagal afferents that can respond to immune mediators such as TNFα and IL-1β. Vagal afferents project to the dorsal vagal complex and from there to varied cerebral regions. (*2*) Humoral route: circulating TNFα, IL-1β, and IL-6-IL-6R complex interact with their receptors on CECs to induce their respective signaling pathways. They can also access the brain via the CVOs and the choroid plexus, regions of the brain that lack a blood–brain barrier. (*3*) Cell mediated route as delineated in mice with liver inflammation: CECs are activated to express adhesion molecules such as P-selectin and VCAM-1. Monocytes in the circulation are activated and express TNFα. TNFα drives increased P-selectin dependent monocyte:CEC adhesive interactions which in turn lead to microglial activation. Activated microglia can produce neuromodulators such as TNFα and NO that can effect neural activity and thereby behavior. In addition, activated microglia express monocyte chemoattractants such as CCL2 which promote monocyte migration into the brain. Infiltrating monocytes also produce CCL2 that can further promote their recruitment into the brain. The infiltrating monocytes can serve as source of cytokines such as TNFα and IL-1β which can mediate changes within the brain. *TNFα* tumor necrosis factor-α, *IL-1β* interleukin-1β, *IL-6* interleukin-6, *NO* nitric oxide; *CECs* cerebral endothelial cells, *CVOs* circumventricular organs

1 Periphery-to-Brain Communication Pathways

1.1 Neural Pathway

Visceral organs are innervated by vagal nerve afferents which could represent the initial trigger for detecting inflammation, as they are capable of responding to cytokines. Vagal nerve afferents express cytokine receptors, including the type I IL-1 receptor IL-1R1, and there are macrophages interspersed between vagal fibers that could also respond to cytokines [18]. In LPS-induced peritoneal inflammation, vagal afferent signaling is activated as demonstrated by increased Fos (protein expressed by the immediate early gene c-fos; used as an indicator of neuronal activation) expression in the vagal afferent primary and secondary cerebral projection areas [19]. Normal volunteers, who received a typhoid vaccination to induce acute peripheral immune activation, showed increased activity in the brain (insula and the anterior cingulate cortex; areas of the brain where afferent vagal and spinal interoceptive neural pathways converge) during performance of a high-demand word task [20]. In addition, fatigue levels in these individuals correlated with activity changes measured within these brain regions. Subdiaphragmatic vagotomy blocks the development of sickness behaviors in rodents in response to inflammation induced by intraperitoneal administration of LPS, but not in response to inflammation induced by alternate routes (i.e., intravenously or centrally) [21]. However, chronic inflammatory diseases are typically associated with prolonged low grade, fluctuating increases in tissue and circulating cytokine levels. Therefore, it is possible that neural communication routes may play a more significant role in development of sickness behaviors in early or more acute and robust inflammatory responses.

1.2 Signaling via Cerebral Endothelial Cells

Receptors for TNFα and IL-1β are found on the cell surface of CECs, and on neuronal and glial cells in the brain [22]. Binding of TNFα and IL-1β to these receptors activates NF-kB in CECs and induces production of secondary messengers (e.g., prostaglandins [PGs] and nitric oxide [NO]) which can subsequently promote changes within the brain [23]. NO is produced by nitric oxide synthase (NOS). Constitutive NOS isoforms are present in endothelial cells (eNOS) and neuronal cells (nNOS). The inducible NOS (iNOS) isoform is up-regulated during inflammation, in immune cells (e.g., macrophages) and CECs, and is associated with modulating CNS function and thereby behavior [24]. For instance, in mice with liver inflammation, iNOS expression was up-regulated in CECs in response to systemic TNFα signaling and was important in mediating microglial activation [5]. Notably, intraperitoneal administration of an iNOS inhibitor in mice exposed to chronic mild stress led to a decrease in cortex iNOS mRNA levels and an

improvement in "depression-like" behavior [25]. PGE_2 is the PG subtype that has received the most attention in its ability to mediate central effects in response to circulating cytokines [26]. PGE_2 receptors are expressed in brain areas implicated in emotional and behavioral control, including the hypothalamus and amygdala [27]. Moreover, inhibition of PG synthesis attenuates systemic LPS-induced sickness behaviors in mice [28].

IL-6R is not expressed on CECs but can be found on leukocytes. When immune cells are activated, IL-6R is shed from the cell surface. IL-6 can bind to the membrane or soluble form of the IL-6R, and the IL-6-IL-6R complex then interacts with the transmembrane component glycoprotein 130, which is expressed on CECs; a process called trans-signaling [29]. IL-6 signaling involves activation of Janus kinases—signal transducer and activator of transcription (STAT) signaling pathway which can induce production of adhesion molecules such as intercellular cell adhesion molecule-1 [23]. In mice with liver inflammation, genetic absence of IL-6 is associated with significant improvement in fatigue-like behaviors, an effect that could be reversed by intravenous administration of recombinant IL-6 [30]. In addition, in mice that underwent a BDL to induce liver inflammation, deficiency in IL-6 prevented the increase in CEC p-STAT3 expression observed in the hippocampal region, highlighting a potential key role for circulating IL-6 in signaling the brain via CECs [30].

1.3 Signaling via the Circumventricular Organs

Most of the brain is enclosed within a blood–brain barrier that is comprised of non-fenestrated endothelial cells with tight junctions between them. CVOs are regions of the brain that lack an intact blood–brain barrier. The capillaries in these regions are fenestrated allowing molecules within the circulation to have direct access to the brain [31]. CVOs may be an access point for circulating cytokines to enter the brain and induce downstream signaling events important in altering brain function. Induction of c-fos mRNA in response to systemic TNFα has been observed in the CVOs [22]. Systemic IL-1β or TNFα administration resulted in increased expression of CCL2 (a potent monocyte chemoattractant) and TNFR mRNA, respectively, within the CVOs [22, 32]. The CVOs have also been implicated as a route for leukocyte entry into the brain. Consistent with this, in brain sections from mice with experimental autoimmune encephalitis, $CD45^+$ leukocytes were observed in four CVOs that were examined (i.e., the area prostrema, organum vasculosum of the lamina terminalis, median eminence, and subfornical organ) [33]. Similarly, in brain sections from mice with liver inflammation, we have also documented infiltrating monocytes in the subfornical region which could suggest that the CVOs serve as a potential route of entry into the brain for circulating monocytes [34].

1.4 Peripheral Immune-Cell-to-Brain Signaling

More recently, we have delineated a peripheral immune cell-to-brain communication pathway that has implications for mediating fatigue-like behaviors and mood disorders in systemic inflammatory diseases [5, 34]. Systemic inflammation is often associated with activation of CECs and circulating leukocytes. This occurrence can therefore result in increased adhesive interactions between them. Circulating monocytes in rodents have been described as a non-inflammatory subtype defined as $CCR2^{-}$, $Ly6C^{-}$, and CX_3CR1^{high} expressing cells, that can give rise to tissue resident macrophages, and an inflammatory subtype, defined as $CCR2^{+}$, $Ly6C^{+}$, and CX_3CR1^{low} expressing cells. Similar monocyte subtypes have been characterized in humans [35]. In mice with liver inflammation, there is a significant increase in the number of circulating inflammatory $Ly6C^{+}$ monocytes (unpublished observation). Moreover, a large proportion of circulating monocytes in mice with liver inflammation are activated and produce TNFα [36]. According to the classical leukocyte recruitment paradigm, leukocytes first tether, then roll along endothelium and upon encountering an activating stimulus, firmly adhere to vascular endothelium [37]. Rolling is primarily mediated by molecules called "selectins." There are three known selectins, namely, P, E, and L-selectin, that can all bind to P-selectin glycoprotein ligand-1 (PSGL-1), a ligand expressed on monocytes. Leukocyte adhesion is mediated by "integrins" which are heterodimers comprising of an "α" and a "β" subunit and are categorized based on a common "β" subunit.

Intravital microscopy is a powerful imaging tool that allows for visualization of leukocyte:endothelial cell interactions occurring within the vasculature in real time. Intravital microscopy of the cerebral vasculature in mice with liver inflammation has demonstrated that peripheral organ inflammation can drive endothelial-immune cell interactions at sites remote from the inflamed tissue. In mice 5 days after liver inflammation induction, cerebral intravital microscopy delineated an increased number of monocyte:CEC adhesive interactions [5]. However, there was no evidence that monocytes were infiltrating the brain parenchyma at that time point. Furthermore, fatigue-like behaviors and altered central neural excitability were observed in these mice [5]. Although monocytes were not infiltrating the brain in mice with liver inflammation at the day 5 time point, microglia (the resident immune cells of the CNS) were activated and expressed TNFα and CCL2. In addition, microglia with an activated morphology were found predominantly in areas around the ventricles and blood-vessels; areas that serve as potential routes of entry for circulating immune cells into the brain [34]. The increased monocyte:CEC adhesive interactions observed in these mice were important for driving subsequent microglial activation, since inhibition of monocyte:CEC adhesive interactions with an anti-P-selectin antibody administered intraperitoneally, significantly reduced microglial activation [5]. In addition, inhibition of monocyte:CEC adhesive interactions in mice with liver inflammation improved fatigue-like behaviors and reversed the central neural excitability changes seen in these mice. In a different animal model, i.e., in a mouse model of pilocarpine-induced seizures, P-selectin

driven neutrophil:CEC adhesive interactions were also found to modulate central neural excitability [38]. While there are limited clinical studies examining behavioral effects of systemic anti-P-selectin therapy, anti-adhesive therapies that target α_4 integrin have been used to treat patients with a range of medical conditions. Natalizumab targets the α_4-integrin chain which blocks leukocyte:CEC adhesive interactions in multiple organs including the gut and CNS. Patients with multiple sclerosis treated systemically with natalizumab reported improvements in fatigue and well-being within 6 months of therapy [39]. Furthermore, in a study that examined effects of natalizumab on fatigue and depression over 1 year treatment, natalizumab treatment in patients with multiple sclerosis was found to significantly improve depression, which in turn was significantly related to improved fatigue [40]. In addition, patients with Crohn's disease treated with natalizumab reported improvement in HRQOL at 12 weeks after starting therapy and showed higher remission rates than placebo-treated patients [41]. However, natalizumab treatment has also been associated with adverse side effects including the devastating neurological disease progressive multifocal leukoencephalopathy. A new anti-adhesion antibody called Vedolizumab has recently been developed. Vedolizumab targets the β_7 chain which forms a heterodimer with the α_4 chain. Hence, Vedolizumab selectively inhibits the recruitment of leukocytes to the gut where the $\alpha_4\beta_7$ integrin is mainly expressed [42]. Vedolizumab treatment has also been reported to improve HRQOL, mainly in patients with Crohn's disease, with no adverse effects of progressive multifocal leukoencephalopathy to date [43].

In mice with liver inflammation of longer duration (i.e., day 10), fatigue-like behaviors are more pronounced than at day 5. Moreover, these day 10 mice have further increases in the number of monocyte:CEC adhesive interactions (as documented by intravital microscopy) as well as in the number of microglia that are activated and expressed the chemokine CCL2 [34]. The increase in microglial activation, along with increased monocyte:CEC adhesive interactions in these mice, was associated with an eightfold increase in the number of CCR2- (cognate receptor for CCL2) expressing monocytes that transmigrated into the brain [34, 36]. Monocytes that infiltrated the brain also expressed CCL2, which likely acts in a positive feedback way to promote further monocyte recruitment into the brain. Cerebral monocyte infiltration was prevented in mice with liver inflammation that lacked CCL2 or CCR2 [34], as well as in mice treated peripherally with anti-P-selectin and anti-α_4-integrin antibodies. Infiltrating monocytes in mice with liver inflammation were found predominantly in the motor cortex, hippocampus, and basal ganglia regions; areas of the brain known to be involved in control of behavior [34]. In addition, monocytes infiltrating the brains of these mice expressed TNFα, a neuromodulator which can promote activation of microglia as well as activate signaling cascades that can affect central neural activity [44]. Microglial activation and CCL2 driven cerebral infiltration of monocytes have also been observed in a mouse model of social defeat stress [45]. Furthermore, inhibition of cerebral monocyte recruitment in mice subjected to social defeat prevented anxiety and depression-like behaviors that classically develop in these mice [45, 46]. Clinical evidence from patients with depression also lends support for a potential role for a

monocyte-to-brain communication pathway in mediating changes in brain function. Increased numbers of perivascular macrophages, as well as increased CCL2 expression in the dorsal anterior cingulate cortex area (a region implicated in mood disorders), have been documented in brain tissue from suicide victims with depression [47].

Microglia are capable of producing a wide range of inflammatory mediators, including the cytokines TNFα, IL-1β, and IL-6. Microglial activation has been linked to behavioral alterations and changes in the regulation of neural excitability [3, 48]. For instance, in CX_3CR1 (expression in brain is restricted to microglia) KO mice a persistent activated microglial phenotype was observed, along with prolonged social withdrawal and "depression-like" behavior, in response to systemic LPS administration [49]. Animal models have demonstrated how inhibition of microglial activation improves sickness and depressive-like behaviors. For instance, in mice with liver inflammation, inhibiting microglial activation using minocycline prevented changes in central neural excitability exhibited by these mice as well as improved fatigue-like behaviors [5]. Furthermore, administration of minocycline to rodents prevented LPS-induced microglial expression of pro-inflammatory cytokines as well as improved observed sickness and depressive-like behaviors [3]. In the clinical setting, microglial activation has been identified in patients with depression or chronic inflammatory diseases that are associated with a high incidence of mood disorders [50]. In patients with chronic liver diseases such as hepatitis (Hep) C and primary biliary cirrhosis (PBC), where comorbidity with mood alterations are high, evidence for microglial activation has been made using cerebral proton magnetic resonance spectroscopy (used to obtain information about cerebral biochemical metabolites), and positron emission tomography. Elevated levels of cerebral myo-inositol (an intracellular metabolite mainly found in glial cells) and choline (suggestive of cellular infiltration or glial proliferation) have been observed in patients with PBC and Hep C. In addition, increased choline levels in the basal ganglia of patients with PBC were associated with cognitive dysfunction [51, 52]. In a study that used both positron emission tomography (with a radioligand that binds to peripheral benzodiazepine receptor; used as a marker of microglial activation) and cerebral proton magnetic resonance spectroscopy, evidence of microglial activation was observed in the basal ganglia of patients with Hep C [53]. In patients with multiple sclerosis, a condition also associated with high incidence of fatigue and depression, positron emission tomography studies showed increased hippocampal microglial activation which in turn correlated with Beck Depression Inventory scores [54].

2 Changes in Brain Function as a Result of Periphery-to-Brain Communication

While circulating cytokines can mediate their effects on central neural activity through the production of secondary mediators such as PGE_2 or NO, they are also capable of inducing de novo synthesis of cytokines within the brain. For instance, peripheral TNFα administration in rodents up-regulates IL-1β and TNFα expression within the brain [55]. Centrally produced cytokines appear to play an important role in mediating the behavioral effects of acutely administered peripheral cytokines. IL-1β production within the brain was demonstrated to be significant in mediating the effects of peripheral LPS since central administration of IL-1 receptor antagonist, an endogenous inhibitor of IL-1β signaling, reduced the expression of IL-1β, IL-6, and TNFα in the hypothalamus and hippocampus of mice as well as the decrease in food intake observed with systemic LPS administration [56]. Functional cytokine receptors are also found on both glial cells and neurons.

Periphery to brain communication results in changes in central neurotransmission or alterations in brain regions associated with regulation of mood and behavior. Healthy volunteers administered endotoxin have increased fatigue along with changes in glutamate activity within the basal ganglia [57]. The basal ganglia comprise several interconnected nuclei which are involved in motor co-ordination, motivation, and emotional control: caudate nucleus, putamen, globus pallidus, substantia nigra, and subthalamic nucleus. Increased levels of glutamate in the basal ganglia have also been observed in patients with major depression, in association with higher levels of systemic inflammatory biomarkers [58]. Manganese is required for normal cellular function within the CNS. However, increased exposure to manganese is associated with microglial activation and neurological alterations in the basal ganglia and cerebral cortex, which in turn are linked to fatigue as well as cognitive and motor abnormalities [59].

Changes in serotonin (5-hydroxytryptamine, 5-HT), dopamine, GABA, and corticotropin releasing hormone (CRH) neurotransmission are amongst some of the neurotransmitter systems that have been implicated in mediating behavioral alterations during systemic inflammation. 5-HT neurotransmission is involved in a wide variety of behaviors including arousal, sleep-wake cycle, locomotor activity, and mood [60]. Serotonergic cell bodies are primarily located in the dorsal raphe nuclei in the midbrain and project to several regions in the brain, including the hippocampus, hypothalamus, and amygdala. Therapies that target 5-HT neurotransmission are associated with improvement in mood in patients with systemic inflammatory diseases. For instance, administration of selective serotonin reuptake inhibitor type drugs that increase cerebral 5-HT levels improves depression in patients with Hep C [61]. Notably, a pilot study with patients with rheumatoid arthritis treated with a TNFα inhibitor showed a decrease in SERT density (5-HT transporter) as well as an improvement in mental and emotional functioning [62]. Tryptophan is an essential amino acid required for the synthesis of 5-HT. Patients with depression generally have low circulating levels of tryptophan.

Indoleamine 2, 3 dioxygenase (IDO) is an enzyme that is expressed in varied cell types, including macrophages, and can be activated by cytokines including TNFα and IFN-γ [63]. IDO converts tryptophan to kynurenine (thereby making less available for 5-HT synthesis) which is further metabolized to kyneuric acid and quinolic acid, all of which have also been implicated in mediating fatigue or mood alterations [2, 57]. Patients with Hep C show elevations in cerebrospinal fluid levels of kynurenine and quinolic acid which are found to correlate positively with depressive symptoms [64]. Quinolic acid is mainly produced by microglial cells and can modulate glutamatergic neurotransmission. Increased density of quinolic acid positive microglia has been observed in the anterior cingulate cortex of depressed patients [65]. Activity changes (as seen via positron emission tomography) within the cingulate and insula regions of the brain have also been made in subjects administered endotoxin to induce depressed mood [66].

While 5-HT can mediate its effects via several receptor subtypes, two 5-HT receptors have received the most attention in relation to fatigue and depression: $5HT_{1A}$ and $5\text{-}HT_3$ receptors. The $5\text{-}HT_{1A}$ receptor subtype is expressed as two separate populations in the brain. They can be expressed as pre-synaptic autoreceptors in the raphe nuclei as well as post-synaptic receptors in limbic structures and in the neocortex [67]. Stimulation of the midbrain pre-synaptic $5\text{-}HT_{1A}$ receptors results in reduced firing of serotonergic neurons, and thereby reduced release of 5-HT into the synapse. Rats with liver inflammation show increased midbrain $5\text{-}HT_{1A}$ receptor expression [68]. Repeated stimulation of the $5\text{-}HT_{1A}$ receptor with a $5\text{-}HT_{1A}$ agonist (i.e., desensitizes the receptor), which would result in increased 5-HT release into the synapse, reduces "depressive-like" behavior in mice with liver inflammation. Patients with major depressive disorder are reported to have a widespread reduction of $5\text{-}HT_{1A}$ receptors in the brain [69]. Mice with liver inflammation show decreased hypothalamic $5HT_3$ receptor expression [70]. Inhibition of the $5\text{-}HT_3$ receptor improves fatigue-like behavior in mice with liver inflammation as well as depressive-like behavior in mice with diabetes [70, 71]. In the clinical setting, altered serotonergic neurotransmission has been reported in Hep C patients with fatigue [72]. In addition, blockade of the $5\text{-}HT_3$ receptor improves fatigue in patients with Hep C [73].

CRH neurotransmission modulates a broad spectrum of behaviors, including anxiety, fatigue, and appetite control [74]. In mice with liver inflammation, hypothalamic CRH levels are decreased [75, 76]. Reduced neuronal activation in the hypothalamic region in these mice has also been suggested by reduced Fos expression observed in the paraventricular region in response to central administration of IL-1β [77]. CRH has been shown to have behavioral activating effects. Rats with liver inflammation that were infused centrally with CRH showed increased locomotor activity at a concentration that had no effect in control rats, suggesting they have increased central sensitivity to CRH [76]. CRH mediates its biological effects via CRHR1 and CRHR2. Rats with liver inflammation show increased hypothalamic CRHR1 expression and treatment with a CRHR1 antagonist attenuates the CRH induced increase in locomotor activity [76]. An association between polymorphisms in the CRHR2 gene and reduced anxiety and depression has been

reported in patients with irritable bowel syndrome (IBS) [78]. In addition, polymorphisms in the CRHR1 and CRHR2 genes have been linked with major depressive disorder [79].

3 Gut Microbiome, Systemic Inflammation, and Altered Behavior and Mood

3.1 Gut Microbiome and Changes During Inflammatory Diseases

The intestinal microbiota contains roughly 10^{14} bacteria which have a profound influence on human physiology and overall health [80]. In addition to their role in digestion and metabolism, the gut microbiota contributes to the development and maintenance of the intestinal epithelial barrier and can modulate the host immune system [81]. Composition of the gut microbiome is readily changeable by diet, pathogen infection, and ingestion of antibiotics, prebiotics, or probiotics. Changes within the gut microbiome are thought to contribute to the onset and progression of IBD as well as systemic inflammatory diseases such as rheumatoid arthritis, diabetes, and chronic liver disease [81, 82]. There is also high comorbidity with sickness behaviors and depression in patients with inflammatory conditions associated with increased intestinal permeability and altered gut microbiome, as documented in patients with post-inflammatory/infectious IBS and chronic liver disease [83]. Notably, depression was reported to be a robust clinical discriminator between IBS patients with a specific microbiome signature, i.e., high Firmicutes: Bacteroidetes ratio, compared to those with a healthy-like microbiota signature [84]. Altered fecal microbiota composition has also been reported in patients with major depressive disorder, further supporting a potential role linking changes in gut microbiota to behavior and mood alterations [85].

3.2 Gut-to-Brain Communication Can Modulate Behavior

The gut-microbiota-brain-axis has become increasingly recognized for its influence on brain development and function, and thereby behavior. Deficits in the serotonergic neurotransmitter system, alterations in brain derived neurotrophic factor (BDNF), and dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis are some of the main biological parameters associated with systemic inflammation and development of sickness behaviors and depression; all of which can be influenced by the gut microbiome [86]. BDNF is a neurotrophin involved in neurogenesis and regulation of cognitive and emotional behaviors. Studies with germ-free animals have highlighted the importance of the gut microbiota in the normal development of

behavior. For instance, germ-free mice exhibit decreased anxiety-like behavior which can be normalized by early gut microbial colonization. Germ-free mice also have lower BDNF levels in brain areas that contribute to regulation of anxiety and fear, including the amygdala, hippocampus, and cingulate cortex [87]. Probiotics are live microorganisms that are commonly ingested to provide health benefits. In a model of chronic-stress-induced depression, mice fed with *Lactobacillus helveticus* NS8 showed improvement in "depressive-like" behavior and cognitive dysfunction, as well as increased hippocampal BDNF and 5-HT levels [88]. Patients with mood disorders also exhibit lower levels of plasma BDNF. A recent study in patients with depression demonstrated elevation in plasma BDNF levels and improvement in symptoms of depression on treatment with agomelatine, an antidepressant known to modulate BDNF levels [89].

In addition to its role in modulating behavior, 5-HT is an important neurotransmitter within the gut [90]. It regulates gut motility and gastrointestinal (GI) secretion. Central 5-HT production accounts for just 5% of total 5-HT synthesis within the body. The majority of 5-HT production occurs in the enterochromaffin cells in the GI epithelium. Alterations in 5-HT neurotransmission may underlie the high comorbidity between GI disorders and behavior alterations. Gut microbiota are thought to play a crucial role in tryptophan availability and metabolism, which could impact central 5-HT levels. Male germ-free mice show elevated circulating tryptophan levels and increased hippocampal 5-HT turnover [91]. Tryptophan levels in germ-free mice are restored to baseline values following colonization with intestinal bacteria demonstrating the influence of gut microbiota on tryptophan availability. Furthermore, probiotics have been shown to alter 5-HT neurotransmission. For instance, in a model of hepatic encephalopathy, Lactobacillus *helveticus* NS8 ingestion improved "anxiety-like" behavior and cognitive dysfunction, in association with increased 5-HT levels [92].

Pathways of communication between the gut microbiome and the CNS are incompletely understood but appear to involve neural and humoral pathways (Fig. 2). Animal models have demonstrated signaling via vagal afferents to be an important neural route of communication between the gut microbiota and the brain. For instance, the anxiolytic effect of *Bifidobacterium longum* was abolished in mice with colitis that were vagotomized [93]. Furthermore, *Lactobacillus rhamnosus* ingestion was shown to reduce stress-induced "depressive-like" behavior in mice, an effect prevented in vagotomized mice [94]. However, vagus-independent mechanisms can also mediate communication between gut-microbiota and the brain as suggested by observations in mice where the behavior modulating effects of probiotics are still evident in vagotmized mice [95]. The intestinal mucosa has multiple innate and adaptive lymphocyte populations that are present in the steady state [81]. Dendritic cells are present beneath the gut epithelium and extend dendrites between epithelial cells to continuously sample and monitor gut luminal content. Microbes can be recognized by pathogen recognition receptors such as toll-like receptors that are expressed by hematopoietic and nonhematopoietic cells in the gut. Changes within the gut environment that result in activation of resident immune cells can lead to increased production of a number of immune mediators,

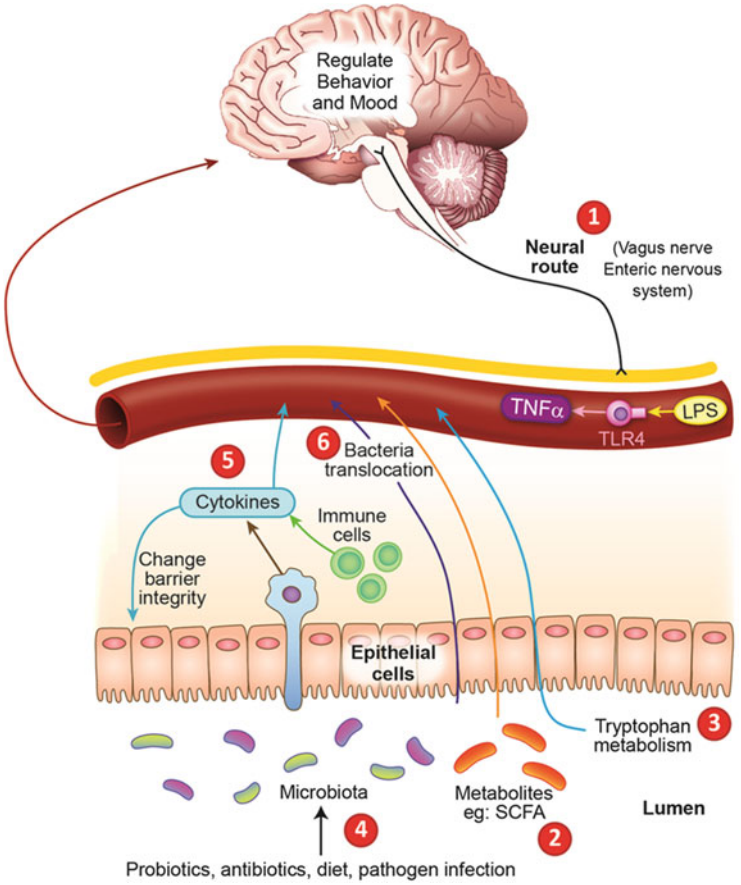

Fig. 2 Gut-to-brain communication (*1*) Neural routes: vagal nerves and enteric nerves can facilitate communication between gut microbiome and brain. (*2*) Gut microbiota produce metabolites such as SCFAs (e.g., acetate, butyrate) via the fermentation of dietary carbohydrates which can have neuroactive properties. (*3*) Majority of 5-HT synthesis occurs within the gut. 5-HT synthesis within the brain is dependent on availability of peripheral tryptophan. Gut microbiota can modulate tryptophan availability in the circulation and thereby effect CNS 5-HT levels. (*4*) Gut microbiome composition is readily changeable by probiotics, antibiotics, diet, or pathogen infection. (*5*) Dendritic cells in the wall of the gut epithelium extend their dendrites between epithelial cells to continuously monitor the gut lumen. Changes within the gut environment that leads to activation of immune cells in the gut can lead to production of cytokines (e.g., TNFα, IFN-γ) that can increase gut inflammation and epithelial cell barrier permeability. (*6*) This would result in a "leaky gut" thereby facilitating translocation of gut luminal bacteria and bacterial products such as LPS into the blood circulation. Circulating LPS can signal via TLR4 on monocytes to mediate production of cytokines such TNFα and IL-1β. These gut-to-brain signaling pathways can lead to alterations in central neurotransmission and thereby behavior changes. *TNFα* tumor necrosis factor-α, *SCFA* short chain fatty acids, *LPS* lipopolysaccharide

including cytokines such as TNFα, which in turn can increase gut epithelial barrier leakage [96]. A "leaky gut" can promote translocation of cytokines and bacterial metabolites such as short chain fatty acids (known to have neuroactive properties) that can either modulate activity of circulating immune cells or gain access to the brain directly via CVOs. Recent work has also demonstrated the ability for the gut microbiome to regulate the permeability of the blood–brain barrier via influencing the expression of tight junction proteins [97]. Furthermore, a "leaky gut" has been demonstrated in depressed individuals through observations of increased bacterial DNA in serum from patients with major depressive disorder, who in turn also showed increased TLR-4 and NF-kβ (part of the TLR-4 signaling pathway) expression in peripheral mononuclear blood cells [98].

3.3 Changes in Gut Microbiome Can Modulate Systemic Inflammation and Thereby Behavior

Probiotic ingestion can have beneficial effects on mood and cognition [99], and can also change neural activity in brain regions involved in emotional processing [100]. However, there are a lack of studies that have examined the effect of probiotic use in depressed individuals. Probiotic consumption has been associated with changes in brain function and behavior in animal models and in patients with a range of systemic inflammatory diseases. For instance, probiotic consumption improved "fatigue-like" behaviors and "depression-like" symptoms observed in rodents after myocardial infarction. The increase in intestinal permeability seen in this model was also reversed by probiotic consumption [101]. In a model of diabetes, probiotic administration was associated with significant improvements in cognitive function and the restoration of impaired hippocampal long term potentiation [102]. Patients with chronic fatigue syndrome, a condition associated with an altered gut microbial profile, showed decreased anxiety after ingesting *Lactobacillus casei* for 2 months [103]. Patients with IBS or rheumatoid arthritis that consumed *Bifidobacterium infantis* 35624 showed significant improvement in quality of life and daily functioning [104–106].

Changes in crosstalk between intestinal epithelium, the intestinal immune system, and gut microbes have increasingly been recognized for its capacity to modulate systemic immunity [107]. As a result of this, probiotics have been administered in an attempt to beneficially alter systemic immunity. For instance, administration of *Bifidobacterium infantis* to patients with IBS was associated with an improvement in symptoms, and a normalization of the IL-10 to IL-12 cytokine ratio in peripheral blood mononuclear cells [106]. VSL#3 is a potent probiotic preparation containing eight live, freeze dried bacterial species (*Streptococcus salivarius* subsp., *thermophilis, Bifidobacterium* [*B. breve, B. infanti, B. longum*], *Lactobacillus acidophilus, L. plantarum, L. casei*, and *L. delbrueckii* subsp. *Bulgaricus*). In the clinical setting, VSL#3 administration is associated with

improvement in QOL in patients with advanced liver disease [108]. We found that administration of VSL#3 to mice with liver inflammation led to significant improvement in "fatigue-like" behaviors [109]. This effect was independent of changes in the severity of liver injury, indicating that the reduction in sickness behaviors seen with VSL#3 treatment was not simply due to changes in liver inflammation. Moreover, mice with liver inflammation treated with VSL#3 had reduced levels of circulating TNFα [109], and a reduction in TNFα production from circulating monocytes (unpublished data). Clinically, ingestion of probiotics, including VSL#3, has been shown to alter circulating levels of systemic pro-inflammatory biomarkers, including TNFα levels, in patients with a range of systemic inflammatory conditions including psoriasis [110], rheumatoid arthritis [111], chronic fatigue syndrome and liver disease [112, 113]. These observations strongly suggest that altering the gut microbiome with probiotics can alter systemic immunity, which in turn can modulate communication between the peripheral immune system and the CNS. The reduction in circulating TNFα levels documented in VSL#3 treated mice with liver inflammation was in turn linked to decreased microglial activation, monocyte:CEC adhesive interactions, and cerebral monocyte infiltration [109]. Importantly, VSL#3 treatment-induced reductions in circulating TNFα levels have also been associated with improved clinical neuropsychiatric outcomes, as observed in patients with chronic liver disease [113].

3.4 Gut Dysbiosis in Systemic Disease

Changes within the gut microbiome are thought to contribute to the pathophysiology of a range of diseases which include GI-related disorders (e.g., IBD) as well as non-GI-related disorders (e.g., obesity, type 2 diabetes) [81]. The use of high-throughput DNA based pyrosequencing technology now enables us to classify bacteria and archaea according to individual 16S rRNA sequences directly from human samples. This has greatly facilitated the ability to obtain detailed profiles of complex communities of microorganisms [114, 115]. Such intestinal microbiota profiling studies have revealed dysbiosis in individuals with specific diseases compared to healthy individuals. For instance, patients with IBD typically show decreased abundance of bacterial species of the phylum Firmicutes and an increase in Bacteroidetes [114]. Obesity, on the other hand, is associated with higher levels of Firmicutes and decreased levels of Bacteroidetes (although this is not consistently found amongst studies) [116]. Furthermore, certain microbial signatures (i.e., high Firmicutes: Bacteroidetes ratio) have been associated with higher incidence of depression in patients with IBS [84]. Intestinal microbiome metagenomic and metaproteomic studies [115, 117] may eventually allow us to use specific changes in microbial composition or function as biomarkers for specific diseases and may identify groups of patients that may be more susceptible to behavioral changes such as depression. Notably, in addition to reducing systemic levels of pro-inflammatory cytokines (e.g., TNFα) and systemic inflammatory biomarkers such as C-reactive

protein (CRP), probiotics are associated with improvement in sickness behaviors [118]. Intestinal microbial profiling studies may potentially identify patients with specific microbial signatures that may show improvement in inflammation-associated behavioral symptoms with probiotic supplementation.

4 Concluding Remarks

Altered behaviors, including fatigue, depressed mood and anxiety, social withdrawal, and cognitive impairment ("brain fog"), are common complaints amongst patients suffering with chronic inflammatory disorders; significantly negatively impacting patient quality of life. Moreover, these complaints often are poorly associated with disease activity and can persist even after diseases are placed into remission through therapeutic interventions. As a result, there is growing interest in developing approaches for treating symptoms associated with chronic inflammation that may (1) target the specific disruption of signaling pathways from the periphery to the brain, which in turn drives inflammatory disease-associated changes in brain function, or (2) modulate altered neural communication processes that develop within the brain in association with chronic inflammation, by targeting changes identified in central neurotransmitters and neurotransmission. Managing disease-associated symptoms, as well as the disease process itself, highlights a growing desire in healthcare to care for patients using a truly holistic approach. Further insights gained from experimental studies in this area, as outlined in this chapter, will facilitate achieving this ultimate goal.

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Mechanisms of Inflammation-Associated Depression: Immune Influences on Tryptophan and Phenylalanine Metabolisms

Barbara Strasser, Barbara Sperner-Unterweger, Dietmar Fuchs, and Johanna M. Gostner

Abstract Metabolic parameters have a direct role in the regulation of immune cell function. Thereby the inflammation-induced metabolism of aromatic amino acids, most importantly of tryptophan and phenylalanine, plays a central role. In addition, neuropsychiatric conditions that go along with disorders that are characterized by acute or chronic inflammation, such as the development of depression, decreased quality of life or cognitive impairments, are connected to disturbed amino acid and subsequent neurotransmitter metabolism.

The bioanalytical procedures for the determination of concentrations of tryptophan and phenylalanine and their respective first stable intermediates kynurenine and tyrosine as well as some analytical finesses and potential sources of errors are discussed in this chapter. Monitoring of these immunometabolic parameters throughout therapies in addition to biomarkers of immune response and inflammation such as neopterin can be useful to determine disease progression but also to plan psychiatric interventions timely, thus to establish personalized treatments.

Keywords Indoleamine 2,3-dioxygenase (IDO-1) • Kynurenine • Neopterin • Phenylalanine • Tryptophan • Tyrosine

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Contents

1 Introduction

The prevalence of psychiatric disorders like anxiety and major depressive disorders has increased during the past decades. Such disorders often accompany chronic inflammatory diseases, but are also associated with older age and loss of cognitive function, especially in the elderly. Although the exact mechanisms that underlie the development of these symptoms are not yet fully explored, it has become obvious that disturbed neurotransmitter biochemistry plays a crucial role. Both of the two most important pathways of neurotransmitter biosynthesis are critical: (1) the serotonergic pathway, which includes the biosynthesis of serotonin from tryptophan, as well as its further conversion to the sleep hormone melatonin; and (2) the dopaminergic, noradrenergic, and adrenergic pathways, which originate from the common intermediate L-3,4-dihydroxyphenylalanine (L-DOPA) and include the synthesis of dopamine, adrenaline, and noradrenaline. Formation of L-DOPA is dependent on the precursor molecules phenylalanine and tyrosine (Fig. 1).

The importance of these neurotransmitter pathways in the pathophysiology of major depressive disorder is the reason why current treatment strategies mainly rely on the application of selective serotonin reuptake inhibitors (SSRI), and on the more recently introduced selective noradrenaline reuptake inhibitors (SNRI), administered alone or in combination. Still, only a subgroup of patients responds favorably to treatment, and it is questionable whether treatment choices based on the measurement of distinct metabolites of theses pathways could increase the therapeutic benefits. Interestingly, biosynthesis of serotonin as well as of L-DOPA derivatives out from the essential amino acids tryptophan and phenylalanine requires the same cofactor, 5,6,7,8-tetrahydrobiopterin (BH_4).

During recent years it has become more and more evident that a relationship exists between specific immune system pathways and neurotransmitter metabolism. Several immunopathological conditions like infections, cancer, and autoimmune syndromes are accompanied by neuropsychiatric symptoms such as fatigue, depressed mood, and cognitive impairment [1–4]. Likewise, similar symptoms

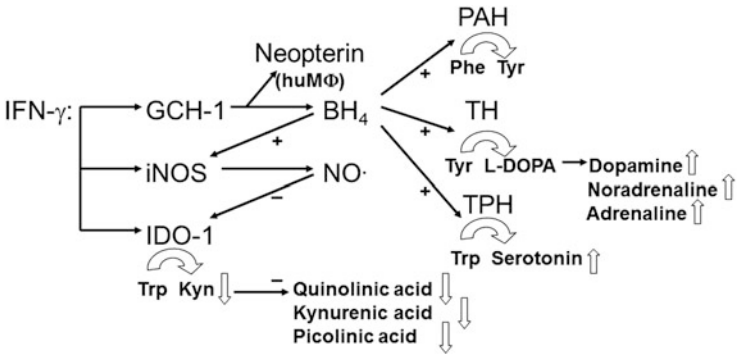

Fig. 1 Influences of immune activation on neurotransmitter biosynthesis in humans. During acute innate and adaptive immune responses, interferon-γ (IFN-γ) induces enzyme GTP-cyclohydrolase 1 (GCH1), which initiates the production of 5,6,7,8-tetrahydrobiopterin (BH_4) in most cells except monocyte-derived cells that produce neopterin. In parallel, several other enzymes like indoleamine 2,3-dioxygenase1 (IDO-1) and inducible nitric oxide ($NO^{\cdot}$) synthase (iNOS) are activated. BH_4 is a cofactor of phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), and tryptophan hydroxylase (TPH) that are important for the biosynthesis of dopamine, noradrenaline, and adrenaline, as well as serotonin. IDO-1 initiates the breakdown of tryptophan (Trp) via the kynurenine (Kyn) pathway. $NO^{\cdot}$ suppresses activity of IDO-1. *L-DOPA* L-dihydroxyphenylalanine, *Kyn* kynurenine, *Trp* tryptophan, *Tyr* tyrosine

can develop in patients during treatment with immune stimulatory cytokines [5], indicating that activation of the immune system is likely to increase the risk of developing neuropsychiatric symptoms. However, this does not necessarily mean that in turn depressed mood feeds chronic inflammation as it was proposed frequently by various authors [6, 7]. Immune activation and depressive mood may feed each other and thus may lead a vicious cycle.

This chapter discusses the interactions between immunological cascades and biochemical pathways of tryptophan and phenylalanine turnover and the usefulness of their assessment in different pathologies, including neuropsychiatric disorders.

2 5,6,7,8-Tetrahydrobiopterin as Cofactor of Neurotransmitter Biosynthesis

The pteridine derivative 5,6,7,8-tetrahydrobiopterin (BH_4) is a cofactor for several monooxygenases such as phenylalanine 4-hydroxylase (PAH, EC: 1.14.16.1), tyrosine 3-monooxygenase (TH, EC: 1.14.16.2), and tryptophan 5-hydroxylase (TPH; EC: 1.14.16.4) (reviewed in [8]). The key role of PAH in the conversion of phenylalanine to tyrosine had been established since the 1970s. After its conversion from phenylalanine, tyrosine is further converted to L-DOPA, the precursor of dopamine, adrenaline, and noradrenaline. TPH synthesizes 5-hydroxytryptophan, which is decarboxylated to form 5-hydroxytryptamine (5-HT, serotonin). In

addition, nitric oxide synthases (NOSs) (EC: 1.14.13.39) and alkylglycerol monooxygenase (AGMO, EC: 1.14.16.5) are dependent on BH_4 availability. NOSs are required for the production of the gaseous nitric oxide radical ($NO^{\cdot}$) from arginine. AGMO – already described in 1964 as a pteridine-dependent enzyme [9] – catalyzes the formation of hydroxyalkyl-glycerol.

Biosynthesis of BH_4 starts from guanosine triphosphate (GTP) by the enzyme GTP-cyclohydrolase I (GCH1, EC: 3.5.4.16, Fig. 1). Other relevant pteridine derivatives such as neopterin (D-erythro 1′2′3′-trihydroxypropylpterin) and the conjugated species of folic acid such as tetrahydrofolate and various other folates, as well as riboflavin (also named lactoflavin or vitamin B_2) originate from this biochemical pathway [8]. In the normal situation, expression of GCH-1 is upregulated by an increase of phenylalanine [10]. This prevents the accumulation of phenylalanine and its neuropathological consequences, as increased phenylalanine levels in the presence of increased BH_4 are converted to L-DOPA and the neurotransmitters dopamine, noradrenaline, and adrenaline.

A drastic enhancement of GCH-1 activity occurs in response to interferon-γ (IFN-γ). Another regulator of GCH-1 is tumor necrosis factor-α (TNF-α), which superinduces IFN-γ-stimulated GCH-1 activity [11–13]. However, it down-regulates GCH-1 feedback regulatory protein (GFRP) in the absence of IFN-γ. In addition, lipopolysaccharide (LPS) down-regulates expression of GFRP, at least in vitro [14], thus rendering pteridine synthesis independent of metabolic control by L-phenylalanine [8].

Inadequate conversion of phenylalanine by PAH leads to hyperphenylalaninemia. Phenylketonuria (PKU) is a disorder characterized by highly increased phenylalanine levels. It results from an autosomal recessive genetic defect in the PAH enzyme gene on chromosome 12q23.2 and is commonly diagnosed by newborn screening. Whereas hyperphenylalaninemia is defined by phenylalanine concentrations >120 μmol/L, in PKU cases the phenylalanine concentrations are usually more than tenfold higher. Beside the classical form of PKU with an incidence of about 1 in 15,000 births – but widely varying between different geographic regions [15, 16], in 2% of cases a much rarer form of the disease occurs due to a defect in the biosynthesis or recycling of cofactor BH_4 despite normal PAH genetics.

Untreated children with PKU are normal at birth. However, they fail to reach even early developmental milestones and demonstrate progressive impairment of cerebral function. In later life, severe mental retardation is the major clinical problem [17]. Today, all newborns are screened for PKU, and if the disease is diagnosed early, a diet low in phenylalanine enables an affected newborn to grow up with normal brain development. However, because the essential amino acid phenylalanine is also required for protein biosynthesis, the phenylalanine level needs to be strictly monitored. In addition, tyrosine must be supplemented. Alternatively, long-term treatment with BH_4 has been demonstrated to be effective in children with mild clinical phenotypes of PAH deficiency [17].

More recently, it was recognized that a moderate form of hyperphenylalaninemia may also develop in clinical conditions of chronic

inflammation [18, 19]. Oxidative loss of BH_4 in such conditions can reduce the biosynthesis of catecholamines, which may relate to disturbed neurotransmitter pathways in patients.

3 Neopterin and Other Pteridine Derivatives and the Immune Response

An increased production of neopterin during states of immune activation was demonstrated for the first time in 1982 both in vitro in stimulated human peripheral blood mononuclear cells (PBMC) and in vivo in allogeneic kidney transplant recipients during rejection episodes [20]. Subsequently, Th1-type cytokine IFN-γ was elucidated as the primary driving force of neopterin production in human and primate monocyte-derived cells like monocytes-macrophages, dendritic cells, and in certain astrocyte-derived cells [21–23]. However, other pro-inflammatory stimuli like IFN-α and -β or LPS are also able to induce neopterin formation, although to a less extent [12]. As it was already indirectly hypothesized from the initial observation, it could be confirmed that increased activity of GCH-1 was the reason for the increased production rates of neopterin in stimulated PBMCs [24]. In other human not monocyte-derived cells, like fibroblasts and endothelial cells, and in cells from other species, IFN-γ also stimulates GCH-1, but BH_4 is formed instead of neopterin. Neopterin levels remain low in these cells because of the high intrinsic activity of pyruvoyl tetrahydropterin synthase (PTPS). This is particularly the case for murine and rat macrophages, which produce BH_4 to serve as a cofactor for the production of $NO^{\cdot}$ by inducible nitric oxide synthase (iNOS).

The production of $NO^{\cdot}$ is triggered by IFN-γ and it is an important mechanism by which macrophages halt the growth and eliminate intracellular pathogens in various species [25]. However, in human monocyte cells, increased $NO^{\cdot}$ production is hampered even after stimulation with IFN-γ, because neopterin and its reduced sister compound 7,8-dihydroneopterin are produced at the expense of BH_4 [26]. Other human cell types are capable to generate BH_4, and thus to form $NO^{\cdot}$ once iNOS is activated [27]. For the same reason the levels of the $NO^{\cdot}$ metabolites nitrite/nitrate are higher in rodents than in human plasma.

Because neopterin is analytically stable, the measurement of native neopterin concentrations in body fluids has turned out to be a useful strategy to get insight in human immune system activation [28]. Significant correlations between concentrations of neopterin and IFN-γ or of other pro-inflammatory cytokines or their soluble receptors, such as the 75 kDa soluble tumor necrosis factor receptor (sTNF-R75) and the soluble interleukin-2 receptor-α (sIL-2Rα) have been reported [29, 30].

Increased neopterin concentrations are observed in patients suffering from infection with viruses, e.g., human immunodeficiency virus-1 (HIV-1), intracellular bacteria as *Mycobacterium tuberculosis,* and parasites like *Plasmodium falciparum.*

In patients with autoimmune pathologies such as systemic *lupus erythematosus* and rheumatoid arthritis, or with malignant tumors, increased neopterin concentrations in body fluids are also quite common. Moreover, neopterin concentrations are increased in patients with cardiovascular disease [31, 32] and with neurodegenerative disorders like Alzheimer's dementia or Parkinson's disease [33, 34]. Of note, in all clinical conditions mentioned above, higher neopterin concentrations correlate with disease activity and predict more rapid disease progression and death. Interestingly, in neurodegenerative disorders, in most cases neopterin levels were higher in the serum or plasma of patients as compared with cerebrospinal fluid (CSF), indicating a gradient from the serum to the brain rather than vice versa.

Although neopterin has been found to increase cognitive performance in rodents when administered into the lateral ventricle of the brain [35], this does not seem to be the case in humans. For instance, neopterin concentrations are a highly significant predictor of cognitive deterioration in thoracic surgery patients [36].

4 Associations Between Tryptophan Breakdown, PAH Activity, and Neopterin Production

Important enzymes in the oxidative catabolism of tryptophan comprise indoleamine 2,3 dioxygenases (protein names: IDO-1, EC 1.13.11.52, and IDO-2, EC 1.13.11, gene names: *IDO1* and *IDO2*) and tryptophan 2,3-dioxygenase (protein name: TDO, gene name: *TDO2*, EC 1.13.11.11). TDO is mainly located in the liver and its activity is regulated by the tryptophan concentration. By contrast, the IDO-1 enzyme is highly inducible by pro-inflammatory stimuli like IFN-γ and TNF-α in monocyte-derived cells but also in several other cell types such as epithelial cells or fibroblast. All the enzymes mentioned above initiate the degradation of tryptophan along the kynurenine pathway. The rate of tryptophan breakdown can be estimated by measuring of the ratio of kynurenine to tryptophan. An involvement of IDO-1 rather than TDO in tryptophan breakdown can be assumed when Kyn/Trp correlates with the concentrations of neopterin or another biomarker of immune activation. In this case Kyn/Trp allows a good estimate of the enzymatic activity of IDO-1 [37]. Kynurenine is further converted to biologically active metabolites by a series of enzymatic reactions, most of them exert cytotoxic and/or neuroactive properties [38].

It is assumed that the decline of tryptophan due to accelerated breakdown will cause a drop of both total and free tryptophan concentrations, because of the equilibrium between the two compartments. Free tryptophan is not albumin-bound and represents the small fraction ($<10\%$) of circulating tryptophan. Only in this form, tryptophan is freely available for uptake by organs and tissues [39] or for transport via the blood–brain barrier. For the transport of tryptophan into the brain the leucine-preferring L1-system is utilized in competition with the so-called large neutral amino acids (LNAA). The ratio of tryptophan:LNAA determines the

flux of tryptophan into the brain [38] and consequently serotonin biosynthesis. Thus, the serum free not the albumin-bound tryptophan concentration is of relevance [39] and one might discuss whether the determination of free tryptophan would bring some advantage for a more detailed interpretation of changes in tryptophan metabolism and its biological consequences. However, free tryptophan is a labile parameter and its measurement requires freshly isolated and ultrafiltered plasma (or serum), which is not available in most circumstances [40].

The first studies comparing the inducible effect of interferons on IDO-1 in immunocompetent cells were published by research groups led by Hayaishi [41] and Wachter [11, 13, 42]. They already showed that interferons exert diverse influences on different cell types, which were confirmed and analyzed in more detail in several later studies [43, 44]. In addition, studies by Werner-Felmayer et al. [11, 13] showed that spontaneous enzymatic activity of IDO-1 is a rather rare event in tumor cell lines and usually induction by cytokines is required. These investigations already indicated that only very few cells possess the enzymatic machinery to further convert kynurenine to downstream metabolites like anthranilic acid and 3-hydroxy anthranilic acid. Aside from that, in vitro experiments revealed a close correlation between neopterin production and tryptophan breakdown rates in freshly isolated and stimulated human PBMC [42]. Notably, the enhanced production of IFN-γ and neopterin is accompanied by an accelerated tryptophan breakdown rate.

Other cells showed parallel induction of both GCH-1 and IDO-1, but unlike human monocytes-derived macrophages, no relevant accumulation of neopterin occurred while BH_4 was produced. The increase of Kyn/Trp could also be observed during human diseases such as HIV-1 infection [45]. Thereby neopterin levels correlate with Kyn/Trp, and additionally, with concentrations of the kynurenine downstream metabolite quinolinic acid [46]. Similar relationships were observed in patients suffering from malignant diseases. In patients with cardiovascular disorders, not only the enhanced neopterin production but also the increased tryptophan breakdown was a significant predictor of outcome. As it is the case in HIV-1 infection, the higher degree of tryptophan breakdown correlated rather closely with the higher concentrations of immune activation biomarkers such as neopterin in CVD patients [37, 47]. However, in vivo measurement of IFN-γ concentrations in serum or plasma is only possible when patients suffer from diseases in which the process of immune system activation takes place in the blood, thus being a systemic process. In the case of a locally restricted inflammation, IFN-γ levels never reach the circulation in relevant concentrations, as concentrations remain low and the cytokine will be bound to its receptors and internalized. Thus, the diffusion of IFN-γ is restricted and allows sensitive quantification of the cytokine only in the microenvironment. Alternatively, due to the higher stability and diffusion properties, measurement of neopterin is more suitable as indicator of immune activation.

Studies in rodents may lead to somewhat different results as compared with the human situation. The difference that exists between monocytes/macrophages of humans vs. rats and mice regarding their abilities to produce $NO^{\cdot}$, influences tryptophan breakdown rates because $NO^{\cdot}$ is a strong inhibitor of IDO-1 activity

[48]. Therefore in humans, due to the absence of high output production of $NO^{\cdot}$ in stimulated macrophages because of BH_4 deficiency, tryptophan breakdown by IDO-1 can be more easily detected than in other species.

Another less explored effect of $NO^{\cdot}$ is its ability to irreversibly inactivate TPH and thus to interfere with serotonin biosynthesis [49]. The presence of BH_4 could even support this mechanism by stabilizing $NO^{\cdot}$, which acts as an antioxidant by itself. One could speculate that $NO^{\cdot}$-mediated inhibition might be of particular relevance for TPH1, which is expressed in the gastrointestinal tract, where diet-derived $NO^{\cdot}$ can be present in considerable amounts [50–52]. Still, it remains to be investigated under which conditions this reaction is of relevance in vivo and whether it contributes to a negative feedback loop preventing overproduction of peripheral serotonin.

5 Associations Between Diminished PAH Activity and Increased Neopterin Production

From an immunobiochemical perspective, it can be predicted that the induction of GCH-1 by cytokines like IFN-γ does not only lead to an increased formation of neopterin in human monocytes-macrophages, but also lead to the production of BH_4 in other surrounding cells. The increased BH_4 availability could trigger the activity of BH_4-dependent enzymes such as PAH, TH, and TPH, and also iNOS and other NOS isoforms, and AGMO. Consequently in cardiovascular disease patients with high neopterin levels, one should expect to observe vasodilation induced by $NO^{\cdot}$. However, patients with poor prognosis present with high blood pressure. Such observations point to subnormal BH_4 levels and thus subnormal activity of BH_4-dependent enzymes. Accordingly patients with HIV-1 infection usually do not present with increased biopterin concentrations despite their sometimes highly elevated neopterin levels [53, 54]. Based on these pathophysiological considerations, the therapeutic potential of BH_4 administration for patients with cardiovascular disease and high blood pressure has been assessed [55]. However, any beneficial action of BH_4 supplementation may be limited due to the oxidation sensitivity of the molecule [56]. Increased concentrations of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) or superoxide anion (O_2^{-}) that are formed during the immune response and are triggered by IFN-γ [57] in parallel to GCH-1 are typical for chronic inflammatory diseases. This scenario corresponds very well with the observations made in patients suffering from coronary artery disease, in whom an association has been observed between higher concentrations of neopterin and decline of several antioxidants including vitamin C and E, lutein, and zeaxanthine [58]. In a similar way, lower vitamin E concentrations were noted in healthy elderly persons who at the same time presented with higher interleukin-6 (IL-6) and C-reactive protein (CRP) concentrations indicating a lowering of the vitamin with higher inflammation degree [59].

A number of studies reported the occurrence of moderately elevated phenylalanine concentrations and phenylalanine to tyrosine ratios (Phe/Tyr) in patients suffering from chronic conditions with a background of immune activation and inflammation, like HIV-1 infection [60] or cancer [19], but also in healthy elderly [61]. Moreover, Phe/Tyr ratios correlated with neopterin concentrations providing a link between the disturbed phenylalanine biochemistry and immune activation. This is further supported by the finding that patients with hepatitis C virus infection present with an increase of Phe/Tyr under treatment with IFN-α and ribavirin [62, 63]. In addition, the increase of Phe/Tyr, which is indicative of disturbed PAH activity, was related to lower dopamine levels and to the development of fatigue [62]. Interestingly, as previously shown for tryptophan breakdown [32] and lower tryptophan serum levels [64], Phe/Tyr ratios correlated with neopterin levels in patients suffering from coronary artery disease [65].

Because BH_4 concentrations are not easily assessable in patient samples and the available methods are quite laborious, Phe/Tyr determinations can serve as a convenient index of BH_4 availability and thus allow also some indirect conclusion about the activity of the other BH_4-dependent enzymes [66]. In most cases of chronic inflammation associated with high neopterin levels, high Phe/Tyr ratios indicate diminished rather than increased BH_4 production. Thus, when an episode of immune activation with overwhelming production of ROS becomes chronic, antioxidant pools are depleted and oxidative stress develops. At the same time BH_4 availability becomes impaired and phenylalanine and Phe/Tyr increase [67]. This has negative consequences on the downstream biochemical pathways. This scenario is supported by the transient increases (blips) of blood phenylalanine concentrations that occur during infectious episodes in children suffering from atypical PKU and under chronic supplementation with BH_4 [68]. Likewise, BH_4 deficiency has also been described in patients with human rabies infection [69].

6 Association Between Dysregulated Tryptophan and Phenylalanine-Tyrosine Metabolism and Neuropsychiatric Symptoms

In the context of the serotonin theory of depression [70] the discovery of the influence of the immune system on tryptophan metabolism led to the conclusion that this pathway is of great relevance for the development of depression. The original concept claimed that induction of IDO-1 during clinical states of immune activation is associated with an enhanced risk of depression [3]. However, this concept does not imply that every form of depression is related to serotonin or kynurenine disturbances caused by immune system activation.

Significant associations between immune system activation and accelerated tryptophan breakdown have been found in patients with colorectal cancer, with positive correlations between decreased tryptophan levels and lower quality of life

as well as higher fatigue scores [71–74]. In a similar way, significant relationships between activation of the immune system, tryptophan breakdown, and development of fatigue have been reported in lung cancer patients [73, 74] and in patients suffering from Epstein Barr Virus infection [75].

Interestingly, many – if not most – of more recent clinical studies on major depressive disorders rule out an inflammatory background of patients, when study exclusion criteria, e.g., elevated C-reactive protein (CRP) or similar biomarkers are defined. Consequently, it is no surprising not to find inflammation-induced changes of tryptophan metabolism as strong denominator for depression in such studies. Despite these difficulties IDO-1 activity is sometimes regarded as a primary player in depression development. The underlying hypothesis is that degradation of tryptophan by IDO-1 creates a decrease in the bioavailability of this essential amino acid for the synthesis of serotonin. This would fit with the observation of increased Kyn/Trp ratios in the blood of patients. However, higher or lower tryptophan levels in the blood do not necessarily lead to parallel increase or decrease of serotonin availability in brain tissue, because the transport of tryptophan into the brain is mediated by the leucine-preferring LAT1-system with tryptophan competing with other large neutral amino acids (LNAA). The ratio of tryptophan/LNAA determines the flux of tryptophan into the brain and thus serotonin biosynthesis [38]. Furthermore, there is no evidence for a direct correlation between peripheral and central tryptophan levels. Raison et al. [76] demonstrated that despite significant decreases in peripheral tryptophan after IFN-α therapy, CSF tryptophan levels were not affected. However, increases in kynurenine were measurable in both body fluids. Thus, elevated Kyn/Trp ratios in the periphery cannot be interpreted to be directly causal for decreased synthesis of serotonin due to decreased tryptophan availability. However, inflammatory conditions might favor oxidative breakdown along the kynurenine pathway at the expense of serotonin synthesis.

Because neuropsychiatric symptoms are common in elderly subjects, the involvement of dysregulated tryptophan and phenylalanine-tyrosine metabolism in these symptoms has been investigated. Age correlated significantly with circulating concentrations of the immune markers neopterin and CRP and neuropsychiatric symptoms, and the increased inflammation was related to lower tryptophan concentrations and increased kynurenine levels [61]. These findings are suggestive of IDO-1-induced tryptophan catabolism associated with older age [77–79]. Inflammation was also associated with increases in neopterin and nitrite levels as well as phenylalanine concentrations at the expense of tyrosine. Moreover, increased tryptophan catabolism correlated with depressive symptoms like lassitude, reduced motivation, anorexia, and pessimism, whereas markers of GCH-1 activity correlated more with neurovegetative symptoms such as sleep disturbance, digestive symptoms, fatigue, sickness, and motor symptoms. Thus, alterations in the two enzymatic pathways might participate in the pathophysiology of different neuropsychiatric symptoms in elderly persons. In a similar way in breast cancer patients with comorbid depression or state anxiety, Phe/Tyr ratios were higher in patients with depression whereas Kyn/Trp ratios were associated with anxiety [72]. In the

same manner, the administration of IFN-α/ribavirin in patients with HCV infection led to an increase of Kyn/Trp [80] and Phe/Tyr [62, 63], whereas dopamine levels in the CSF were diminished [62] and the decreases in dopaminergic activity were correlated with fatigue scores. These results could form the basis for future treatment studies. In cancer patients with comorbid depression for instance, the use of serotonin-noradrenaline reuptake inhibitors might be recommended while in those with predominant anxiety selective serotonin reuptake inhibitors might be the treatment of choice. In those patients who have mainly fatigue as a symptom the use of drugs targeting dopamine deficiency could be indicated.

During and after pregnancy, women display significantly accelerated tryptophan breakdown that correlates with the gestational week and normalize during puerperium [81]. It was hypothesized that IDO-1 is involved because of the significant relationship between Kyn/Trp levels and neopterin. Following up on these findings, Munn and colleagues demonstrated that IDO-1 activation during gestation in mice was required to achieve maternal immunotolerance against the fetus [43]. Later on, several significant relationships between the tryptophan metabolic alterations and the development of neuropsychiatric symptoms in women were reported. Postpartum blues develops preferentially in those women who present with continuously low serum tryptophan after delivery due to an increased degradation to kynurenine, but this effect is independent of variations in neopterin levels [82, 83].

Tryptophan and serotonin are precursors for the biosynthesis of melatonin that plays an important role in sleep regulation [84]. Sleep disturbance is a strong contributor to poor quality of life and an increased risk of depression development. A link between the enhanced tryptophan breakdown and sleep disturbance has been described in patients with HIV-1 infection [85]. Disturbed tryptophan metabolism may underlie not only poor sleep but also could explain the increased susceptibility to the common cold that has been found to be associated with poor sleep [86].

However, the disturbed tryptophan-serotonin pathway is not the only biochemical route that is relevant for the development of neuropsychiatric symptoms, e.g. the phenylalanine-tyrosine-dopamine pathway and corresponding biogenic amines are of comparable significance. Supplementation with BH_4 should theoretically correct the impaired activity of all BH_4-related neurotransmitter pathways. This concept emerged in the early eighties [87, 88], but early treatment trials were not successful [89]. Several questions regarding selection of patients and treatment modalities, as well as bioavailability still need to be answered. Moreover, BH_4 availability is certainly not the only factor that needs to be taken into consideration for normalizing alterations in neurotransmission, e.g., serotonin receptors certainly play an important role [90]. Another set of critical factors is represented by kynurenine metabolites that act as agonist (quinolinic acid) or antagonist (kynurenic acid) of the *N*-methyl D-aspartate (NMDA) receptor.

7 Immunobiochemical Pathways Influenced by Life Style

Immune status and inflammation can be influenced by life style factors including nutrition and exercise, but also smoking. Tryptophan represents a key element for brain functioning, and nutrients rich in tryptophan like cashew nuts, turkey, or banana, to name a few, are often claimed to exert a positive impact on mood and cognition [91]. However, the actual tryptophan content of such nutrients can only be of limited relevance to brain metabolism because the levels of competing amino acids are high and any benefit of such food to brain pathways via the LAT-1 system (see above) remains questionable. Moreover, because of the complex composition of a diet, it is difficult to define that the effect of a single compound rather multiple compounds may act synergistically to mediate beneficial effects.

Findings derived from the in vitro model of mitogen-stimulated peripheral blood mononuclear cells revealed that several phytochemicals, mainly antioxidants like polyphenols and vitamins, can interfere with inflammatory signaling cascades, which as a consequence may also reduce tryptophan breakdown rates [50, 92]. If extrapolated to in vivo conditions antioxidants could dampen cell-mediated immunity and thus tryptophan breakdown via IDO-1. This would result in an increase of blood and brain tryptophan availability. Although there is some evidence for such in vivo effects of antioxidants, outcomes are hardly predictable and most likely depend on an individual's immunological state [93]. In conclusion, probably more than a diet rich in tryptophan, a diet rich in antioxidants can have a positive impact on sleep, mood, and cognition when the tryptophan-serotonin-melatonin metabolic chain is improved. Thus, dietary interventions could be of greater relevance especially in individuals who present with conditions of low grade inflammation.

However, overwhelming exposure to antioxidant compounds – that can be nutrients with supplemented vitamins, food preservatives, and/or colorants – can cause "anti-oxidative stress."

Excessive intake of antioxidants could play a role in allergy development when the suppression of Th1-type immunity upregulates its counterpart the Th2-type immune response [94, 95]. Interestingly patients suffering from acute allergic responses may present with increased rather than diminished blood tryptophan concentrations [96]. From that point of view, likelihood of depressive mood should be more rare in atopic as compared with non-atopic patients. However, this has not yet been studied.

The positive effects of physical activity on mood and mental disorders are well documented [97]. With regard to mood, most studies focus on the influence of physical activity on depressive symptoms or affective disorders. These studies show that aerobic exercise at a dose consistent with public health recommendations is an effective treatment for mild-to-moderate major depressive disorder [98, 99]. In addition, exercise is associated with long-lasting increases in brain tryptophan levels [100]. A recent study identified a mechanism by which skeletal muscle peroxisome proliferator-activated receptor gamma coactivator 1-alpha 1 (PGC-1α1), induced by exercise training, switches kynurenine metabolism from

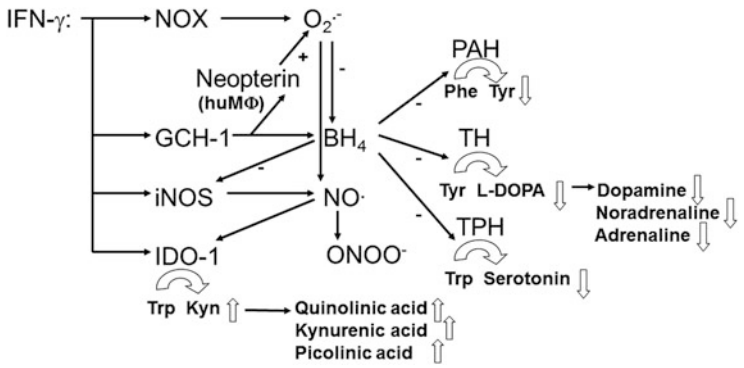

Fig. 2 Chronic activation of the immune system influences neurotransmitter biosynthesis differently from acute inflammation. Prolonged formation of reactive oxygen species like superoxide anion (O_2^-) by NADPH-oxidase (NOX) wipes out antioxidant systems including BH_4. Consequently, the function of BH_4-dependent enzymes is disturbed and the production dopamine, noradrenaline, adrenaline, but also serotonin is diminished. In a similar way, NO· reacts with O_2^- and produces highly toxic vasoconstrictant peroxynitrite (ONO_2^-), causing hypertension. The inhibition of IDO-1 by NO· is no longer active and the formation of the kynurenine pathway metabolites is increased at the expense of serotonin. *L-DOPA* L-dihydroxyphenylalanine, *Kyn* kynurenine, *Trp* tryptophan, *Tyr* tyrosine

quinolinic acid to kynurenic acid production, and protects from stress-induced depression [101]. However, the clinical relevance of these findings remains to be determined.

Physical activity has significant impact on inflammation cascades and induces immunoregulatory cascades involving IFN-γ and other cytokines [102, 103] impacting down-stream biochemical pathways like the production of neopterin. More recently it was demonstrated that amino acid profiles are altered during and after a half iron man triathlon [104]. Such data fit well with the observation that intense training is associated not only with an increase of neopterin, but also with concomitantly accelerated tryptophan breakdown and increased Kyn/Trp [105].

Interestingly, acute moderate physical exercise can have effects opposite to those of chronic physical exercise. Physical exercise may enhance the production of neurotransmitters by induction of BH_4 synthesis, which would accelerate the activities of BH_4-dependent enzymes and result in increases in neurotransmitter biosynthesis (Fig. 1), which could contribute to a heightening of mood. At the same time, production of NO· increases and due to vasodilation blood pressure declines. However, counter-regulatory pathways are initiated in parallel leading to activation of pro-inflammatory cascades and production of ROS, which deplete endogenous antioxidant pools including BH_4 concentrations (Fig. 2). Breakdown of tryptophan by IDO-1 during intense exercise diminishes tryptophan availability that finally slows down serotonin formation.

The oxidation processes further activate the cell's detoxifying machinery, as can be seen by an increase of superoxide dismutase (SOD) -1 and -2 or glutathione peroxidase [106, 107]. Consequently, BH_4-dependent biosynthesis of several

neurotransmitters will decline and the primarily enhanced mood will be followed by a decline that may play a role in the onset of fatigue and sleep disturbances when sports become too heavy or training intervals too short. As a consequence, adherence to training will suffer. The same is true for the production of $NO^{\cdot}$, its production will suffer, too, and no further benefit of physical exercise to lower blood pressure can be expected, rather the vasoconstrictive effects of oxidative stress will become evident [108]. It is assumed that this is similar to the scenario that may take place in acute vs. chronic diseases such as microbial infections in which the antiproliferative activity of the immune system results first in decreased tryptophan. At the same time BH_4 goes up and may partially compensate deficient production of serotonin. However, in the later course also BH_4 becomes deficient as does the production of the related biogenic amine neurotransmitters, and the likelihood of neuropsychiatric symptoms elevates. Thus, there might be a beneficial effect of antioxidant supplements to counteract the negative psychological effects of intense sports [107].

This relationship becomes especially important when exercise is performed as part of a weight-loss program together with a reduction of food intake. After short time, calorie restriction diet is associated with the decline of serum/plasma tryptophan levels followed by a decline of phenylalanine [109]. Consequently, the adherence to a weight loss diet could result in a deficiency of the relevant essential amino acids and one might consider supplementation of these during such periods to avoid such unwanted side effects [110]. Both effects together will improve availability of the neurotransmitters that influence mood.

Finally, composition of gut bacteria is influenced by dietary components and may impact on serotonin metabolism [111]. Not surprisingly, the diet is an important factor for the composition of the human intestinal microbiome [112]. Interestingly, in patients with Alzheimer's dementia, low serum tryptophan [113] but not phenylalanine levels [114] correlate with cognitive performance and are associated not only with the systemic inflammatory status of patients, but also with specific inflammation biomarkers in stool such as calprotectin, which indicates a pathologically increased permeability of the intestinal barrier [115].

Other lifestyle factors can affect aromatic amino acid metabolism. Carbon monoxide (CO) binds to heme iron and impedes oxygen supply. Thus, CO from smoking or air pollution can suppress IDO-1 activity by binding to active site heme [116]. In fact, even low doses of CO exert anti-inflammatory properties and are therefore used in clinical trials for treatment of, e.g., acute lung injury or in sepsis patients [117].

8 Conclusion

The measurements of tryptophan and phenylalanine metabolism can provide reasonable insight into the biochemical pathways involved in the pathogenesis of neuropsychiatric abnormalities, support treatment personalization, and predict

outcome. In parallel, diet can influence immunobiochemical pathways. These pathways are very sensitive to situations of inflammation but can also be modulated to some extent by lifestyle factors including diet.

In the absence of an inflammatory response, plasma kynurenine levels are kept relatively stable by the activity of TDO2 and further downstream catabolism in the liver. During inflammation, activation of the kynurenine metabolism pathway elevates circulating kynurenine levels [118] while circulating tryptophan levels decline. This situation can be different in the CSF, where kynurenine metabolism may behave independent from the periphery.

Because activation of the kynurenine pathway is assessed by measuring circulating levels of tryptophan, kynurenine, and kynurenine metabolites, it is important to note that ways of measuring these metabolites can be misleading. In particular, measurement of plasma/serum kynurenine levels after precipitation of proteins by the use of acidic reagents [119] contributes to their loss especially in the presence of nitrites because of the formation of diazotization products according to the Sandmeyer reaction [120]. This is of greater relevance when measuring samples from mice or rats that have higher nitrite content than human samples. Regarding the measurement of Phe/Tyr, this ratio not only reflects the phenylalanine hydroxylase activity itself, but also provides an approximate for BH_4 availability [19].

As already mentioned, amino acid levels may fluctuate due to various insults. Therefore, it is important that a parallel measurement of inflammation biomarkers is performed to distinguish inflammation-induced IDO-1 activity from TDO. Thereby, neopterin has turned out as a stable and reliable analyte and a correlation between Kyn/Trp and neopterin can serve as strong support of the involvement of IDO-1 rather than TDO in tryptophan breakdown. Still it needs to be considered that cortisol is often released during inflammatory responses and is known to upregulate TDO [121].

In the last two decades, a number of important findings on the involvement of aromatic amino acid metabolism in depression have been obtained. However, still much needs to be worked out to allow a more detailed assessment of their individual contribution and lead to better rationales for the choice of intervention and treatment.

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Role of the Kynurenine Metabolism Pathway in Inflammation-Induced Depression: Preclinical Approaches

Robert Dantzer

Abstract Physically ill patients with chronic inflammation often present with symptoms of depression. Our understanding of the pathophysiology of inflammation-associated depression has benefited from preclinical studies on the mechanisms of sickness and clinical studies on the symptoms of sickness and depression that develop in patients treated with immunotherapy. Sickness behavior develops when the immune system is activated by pathogen- or damage-associated molecular patterns. It is a normal biological response to infection and cell injury. It helps the organism to mobilize its immune and metabolic defenses to fight the danger. Depression emerges on the background of sickness when the inflammatory response is too intense and long lasting or the resolution process is deficient. The transition from sickness to depression is mediated by activation of the kynurenine metabolism pathway that leads to the formation of neurotoxic kynurenine metabolites including quinolinic acid, an agonist of *N*-methyl-D-aspartate receptors. The neuroimmune processes and molecular factors that have been identified in the studies of inflammation-associated depression represent potential new targets for the development of innovative therapies for the treatment of major depressive disorders.

Keywords Behavior • Brain • Cytokines • Depression • Indoleamine 2,3 dioxygenase • Inflammation • Kynurenine • Microglia • NMDA receptor • Quinolinic acid • Sickness

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Contents

1 History of a Discovery: From Inflammation-Induced Sickness to Depression

I read with intense excitement the original research article by Munn and colleagues on the prevention of allogeneic fetal rejection by tryptophan catabolism when it came out in *Science* in 1998 [1]. This landmark paper provided a very simple explanation for what had puzzled reproductive immunologists for a long time: how the foreign mammalian conceptus can avoid immune attack and defend itself against rejection by the mother's immune system. Munn and colleagues showed convincingly that maternal T-cell tolerance to the fetal allografts can develop because the maternal immune response to the allogeneic conceptus activates the tryptophan-metabolizing enzyme indoleamine 2,3 dioxygenase (IDO1) in the trophoblast at the level of the maternal–fetal interface. IDO1 metabolizes tryptophan, an amino acid nutrient essential for T-cell proliferation and cytotoxicity, into kynurenine. The resulting depletion of tryptophan leads to T-cell anergy.

I had no special interest in reproductive immunology; my excitement came from the realization that a process similar to the one described by Munn and colleagues could occur in the brains of inflamed organisms and be responsible for some of the effects of cytokines on the brain. We had already demonstrated that systemic inflammation induces the production of proinflammatory cytokines in the brain and that these proinflammatory cytokines cause sickness behavior [2]. If proinflammatory cytokines could activate IDO at the maternal–fetal interface, they could certainly do it in the brain. The result would be a decrease in the bioavailability of tryptophan for the synthesis of serotonin, which could account for why inflammation is associated not only with sickness behavior but also with major depressive disorders.

It took us some time and effort to test this hypothesis. Thanks to a collaboration with Maes, who was then at the University of Maastricht in the Netherlands, we already had evidence that circulating levels of tryptophan were decreased in cancer patients treated with interleukin (IL)-2 or interferon (IFN)-α [3]. What was striking in these findings was that the severity of symptoms of depression was positively correlated with the magnitude of the decrease in tryptophan concentrations during

the treatment. However, in order to test the hypothesis that activation of IDO is responsible for the development of inflammation-induced depression, we first had to show in mice that systemic activation of the immune system activates IDO in the brain and then that the time course of this response is compatible with the time course of the development of symptoms of depression.

There were several hurdles to jump over before this demonstration could be achieved. First, most of our work on the brain effects of cytokines had been done in rats. However, IDO activation in microglia and astrocytes in response to systemic or central inflammation was already known to be minimal in the rat [4], probably because this species responds to systemic inflammation by a strong induction of nitric oxide synthase, with the resulting nitric oxide opposing IDO activation [5]. We therefore had to switch to a mouse model [4]. Second, we had to take into account the fact that the bioassays for assessing depression in rodents were based on behavioral responses that are very sensitive to sickness. The reduction in sucrose preference that is commonly used to assess anhedonia was going to be biased by the decreased appetite present in sick animals. In the same manner, the increased duration of immobility in the forced-swim and tail-suspension tests, which are used to assess helplessness, was going to be difficult to separate from the decreased motor performance associated with sickness. In other words, there was no way for us to convince reviewers that we could measure depression-like behavior independently of sickness behavior in inflamed mice.

One possibility to circumvent this issue was to wait until sickness dissipated before measuring depression-like behavior. This turned out to be the right strategy, as our time-course studies of IDO activation in the brain revealed that IDO enzymatic activity requires 24 h to develop in the brains of mice injected with lipopolysaccharide (LPS) or superantigen at the periphery [6] (Fig. 1). By that time, sickness behavior measured by reduced food intake, decreased social exploration, and reduced locomotor activity would have fully dissipated and could therefore no longer account for the increased duration of immobility in the forced-swim test and the reduced sucrose preference presented by LPS-treated mice [7]. This dissociation between sickness behavior and depression in response to LPS fitted very well with the already-demonstrated temporal dissociation between the neurovegetative

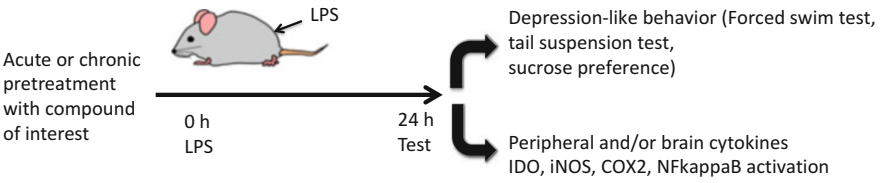

Fig. 1 Experimental design for studying inflammation-induced depression in mice. Mice are injected with a sub-septic dose of lipopolysaccharide (LPS) at time 0, preceded or not by the treatment of interest (e.g., an anti-inflammatory compound). Their sickness behavior develops within a few hours and wanes off by 12–16 h post-LPS. Behavioral tests of depression are carried out 24 h after LPS administration, and tissues are collected immediately afterward to measure biomarkers of inflammation and kynurenine metabolism

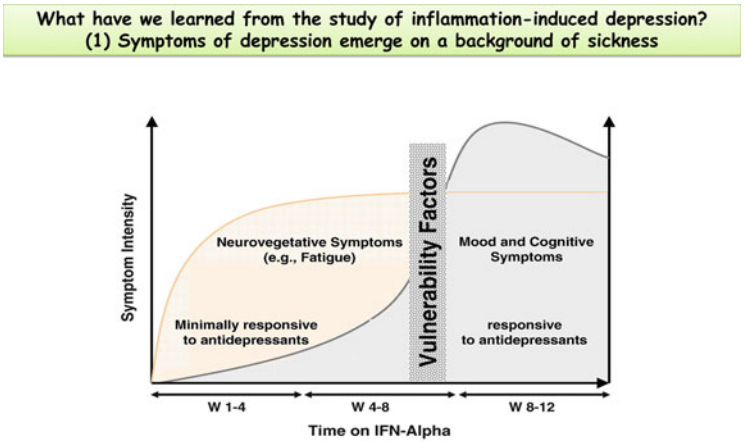

Fig. 2 Time course of the development of symptoms of depression in response to interferon (IFN)-α in patients with cancer. Time is represented in weeks (W). Note that neurovegetative symptoms (including reduced appetite, sleep disorders, and fatigue) emerge first in response to repeated injections of IFN-α, whereas mood and cognitive symptoms emerge later. IFN-α-induced symptoms of depression differ in their response to antidepressant treatment. Neurovegetative symptoms appear in all patients, whereas mood and cognitive symptoms appear only in one third to one half of patients who have vulnerability factors, represented, for instance, by single nucleotide polymorphisms of proinflammatory and anti-inflammatory genes (adapted from [9])

symptoms and the cognitive/affective symptoms of depression seen in patients treated with IFN-α [8] (Fig. 2).

The only methodological problem left was the relatively short duration of LPS-induced depression, which did not last for more than a few hours. We therefore needed to set up a model of chronic inflammation-induced depression. Because the main driver of IDO activation is IFN-γ, we selected an IFN-γ-inducer pathogen represented by Bacillus Calmette-Guerin (BCG), an attenuated form of *Mycobacterium bovis,* to activate IDO chronically. We confirmed that mice inoculated with BCG had chronically elevated IDO activity in the lung and brain [10]. We were also able to demonstrate that the initial episode of sickness that developed a few days after BCG inoculation was followed by long-lasting decrease in sucrose preference and increase in immobility in the forced-swim and tail-suspension tests [11].

On the basis of these results, we were able to move to the next step in this research and demonstrate that IDO activation is crucial for the transition from sickness behavior to depression-like behavior in both LPS-treated and BCG-treated mice [12–14]. This allowed us to investigate the mechanism responsible for inducing depression downstream of IDO. This chapter will show how the immune-mediated tryptophan depletion hypothesis of inflammation-induced depression has been ultimately replaced by the kynurenine metabolism hypothesis, and will discuss how this mechanism can be targeted for treating inflammation-induced depression.

2 From Tryptophan Starvation to Neurotoxic Kynurenine Metabolites

Most of the tryptophan we ingest is metabolized along the kynurenine pathway, and only a tiny amount, about 1%, is converted into serotonin (Fig. 3). The conversion of tryptophan into N-formyl kynurenine is catalyzed by the liver enzyme tryptophan 2,3 dioxygenase (TDO), also known as tryptophan pyrrolase. IDO is the product of the *ido1* gene and is sometimes labeled as IDO1. Another IDO-like enzyme was recently discovered. It is the product of a different gene, known as *ido2*, that is situated on the same chromosome as *ido1* and that probably emerged through gene duplication. This enzyme was accordingly labeled as IDO2. It also has the ability to metabolize tryptophan, although its enzymatic activity is much lower than that of IDO1. Its physiological and pathophysiological roles are still unclear [15]. In this chapter we will refer only to IDO1, which we will continue to label as "IDO."

IDO was first purified from the rabbit intestine and found to have a broader substrate specificity than did TDO [16]. Yoshida and Hayaishi from the Department of Medical Chemistry at the Faculty of Medicine in Kyoto were the first researchers to show that LPS injected intraperitoneally induces IDO in the lungs, peaking 24 h after injection [17]. The same effect was obtained after viral infection [18]. IFN-γ mimics the effect of LPS and viral infection on induction of IDO, and cloning of the *ido1* gene confirmed the ability of IFN-γ to upregulate IDO [19]. IFN-γ and other cytokines increase the transcriptional activation of *ido1*. More specifically, IFN-γ-induced signal transducer and activator of transcription 1α (Stat1) activates

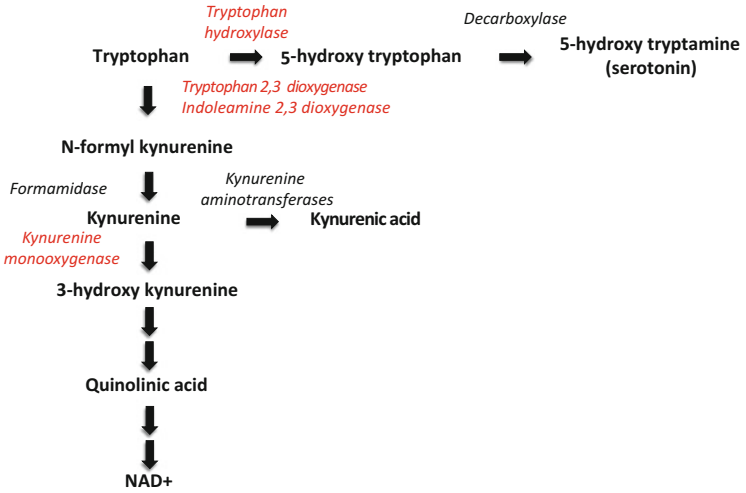

Fig. 3 Schematic representation of the tryptophan, kynurenine, and serotonin metabolism pathways. The enzymes for which the activity is modulated by inflammation are labeled in *red*. Pathways represented by *two arrows* involve several metabolites and enzymatic reactions

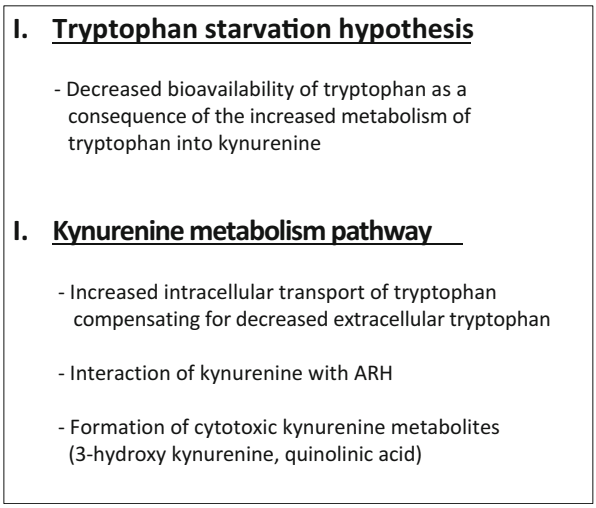

Fig. 4 The various hypotheses proposed to account for the effect of activation of indoleamine 2,3 dioxygenase and the kynurenine pathway on immune functions

ido1 gene expression by binding to γ-activated sequences in the *ido1* regulatory region. In addition, IFN-γ induces IFN regulatory factor-1, which binds to IFN-γ-stimulated response elements (ISRE) in the *ido1* regulatory region. Tumor necrosis factor (TNF)-α synergistically increases the transcriptional activity of *ido1* in response to IFN-γ by increasing Stat1 and ISRE [20].

Activation of IDO leads to enhanced metabolism of tryptophan into kynurenine, which decreases the bioavailability of tryptophan for its metabolic functions. IDO-induced tryptophan starvation has long been believed to be responsible for the metabolic control of immune responses [21] (Fig. 4). Tryptophan starvation that develops in the inflammatory microenvironment triggers amino-acid-sensing pathways and in particular the serine/threonine protein kinase general control nonderepressible 2. This leads to enhanced generation of Foxp3+ regulatory T cells and inhibition of T effector cells. Tryptophan starvation also inhibits mechanistic target of rapamycin (mTOR) activity, leading to the same results.

The importance of tryptophan starvation in the biological consequences of IDO activation has recently been questioned. Tryptophan remains available in the extracellular milieu and in the blood in relatively high concentrations during IDO activation, and most cells are able to incorporate tryptophan with high efficiency even in conditions of low tryptophan availability. The attention has therefore shifted to the possible activity of kynurenine generated from tryptophan by IDO and the kynurenine metabolites generated by further enzymatic reactions (Fig. 3).

In contrast to what was initially believed, kynurenine is not biologically inactive. It acts as a high-affinity ligand of the aryl hydrocarbon receptor (AHR). AHR is present in many different cells and plays an important protective role at the level of mucosal and barrier tissues [22]. Activation of AHR by kynurenine has immunosuppressive properties on innate and adaptive immunity. Binding of kynurenine to AHR downregulates inflammatory responses mediated by LPS acting on

macrophages and promotes endotoxin tolerance [23,24]. In the presence of transforming growth factor-β, kynurenine reduces the differentiation of T cells into highly inflammatory Th17 cells and promotes the generation of Foxp3+ regulatory T cells [25]. In addition to its biological activity, kynurenine is metabolized into kynurenine metabolites by additional enzymatic reactions. In particular, kynurenine is converted by kynurenine aminotransferases into kynurenic acid and by kynurenine mono-oxygenase and other enzymes into 3-hydroxy kynurenine, 3-hydroxy anthranilic acid, and quinolinic acid (Fig. 3). These last kynurenine metabolites have additive cytotoxic effects on T lymphocytes [26].

Two independent lines of research have dominated the studies on tryptophan and tryptophan metabolism in the central nervous system. The first line of research finds its origin in the early 1970s and focuses on the role of tryptophan in the synthesis of serotonin [27]. The second line of research focuses on the physiology and pathophysiology of kynurenine and its metabolites in the brain [28]. Concerning the first line of research, two nutritionists from Massachusetts Institute of Technology, Fernstrom and Wurtman, reported that it was possible to modify brain concentrations of serotonin in rats by administering L-tryptophan at the periphery [29]. This modification was possible with as little as 12.5 mg/kg tryptophan, which is much less than the amount of tryptophan rats normally consume daily in dietary protein. Because of this, Fernstrom and Wurtman proposed that physiological fluctuations in plasma tryptophan concentrations influence brain serotonin levels. Given the observation that tryptophan hydroxylase is not normally saturated by the physiological concentrations of its substrate, it was theoretically possible to increase brain serotonin by administering its precursor at the periphery and ultimately to treat disease states related to reduced brain serotonin [30]. Curzon at the London Institute of Neurology proposed a link between major depressive disorders and enhanced degradation of tryptophan along the kynurenine metabolism pathway because of an increased activity of TDO in response to elevated endogenous cortisol levels [31,32].

Despite all these favorable elements, tryptophan supplementation in humans never held to its promises. Ingestion of tryptophan did not improve mood and at best induced some increase in drowsiness – but only after severe initial nausea and headache [33]. Other studies using tryptophan supplementation indicated that increased serotonin decreased quarrelsomeness and increased agreeableness in social interactions, therefore acting on mood in a very indirect manner [34]. In contrast, acute tryptophan depletion produced by ingestion of a mixture of branched-chain amino acids other than tryptophan that competed with tryptophan for transport into the brain turned out to be an easy way to decrease brain serotonin levels momentarily and lower mood. However, this effect occurred reliably only in subjects with a personal or a family history of depression [34,35].

It is not surprising that the initial hypothesis for the relationship between inflammation and depression focused on decreased serotoninergic neurotransmission. By decreasing circulating levels of tryptophan, inflammation-induced IDO activation has the potential of mimicking the effect of acute tryptophan depletion on serotonin metabolism. In addition, IFN-γ induces guanosine triphosphate

cyclohydrolase I (GTP-CH) in macrophages. GTP-CH is a key factor in the synthesis of biopteridines. Activation of GTP-CH favors the formation of neopterin over that of tetrahydrobiopterin (BH4). BH4 is an important co-factor for the enzymatic activity of tryptophan hydroxylase, which metabolizes tryptophan into 5-hydroxy tryptophan, an essential step in the synthesis of serotonin. However, as mentioned in the first section of this chapter, there is no evidence for decreased brain tryptophan and serotonin levels in inflammation-associated depression. Much of the speculation on GTP-CH has switched to its role in the enzymatic activity of tyrosine hydroxylase and the formation of phenylalanine, a precursor of dopamine (see [36]).

In parallel with the fading of the tryptophan starvation hypothesis in immunology, studies on the mechanisms of inflammation-associated depression began to examine the kynurenine metabolism hypothesis, making use of what was already known about the pharmacology of kynurenines in the central nervous system. Lapin at the Bekhterev Psychoneurological Institute in Leningrad, USSR, had observed that kynurenine and its metabolites (mainly 3-hydroxy anthranilic acid, anthranilic acid, picolinic acid, and nicotinic acid) had anti-serotonin and anti-tryptamine activities [37]. When injected into the brain ventricles of mice, kynurenine, quinolinic acid, 3-hydroxy anthranilic acid, xanthurenic acid, picolinic acid, and nicotinic acid had potent pharmacological activities manifested by increased motor activity and convulsions [38]. Kynurenic acid was found to be able to antagonize the convulsant effect of kynurenine and quinolinic acid [39].

Research on the pharmacology and physiology of brain kynurenines has made great progress since these initial studies [28]. Kynurenic acid is a broad spectrum competitive antagonist of glutamate receptors and an inhibitor of the α7 nicotinic acetylcholine receptor. Quinolinic acid acts as an agonist of *N*-methyl-D-aspartate (NMDA) receptors, mainly in the forebrain. Other metabolites of kynurenine, including 3-hydroxy kynurenine, 3-hydroxy anthranilic acid, and anthranilic acid, have no direct effect on neuronal activity but participate in complex pro-oxidative and anti-oxidative processes. However, most of the studies on the physiology and pharmacology of kynurenine metabolites completely ignored the role of immune-mediated IDO activation and focused primarily on the neuronal effects of these metabolites.

3 Inflammation-Induced Depression and Glutamate Neurotransmission

The possibility of a cellular compartmentalization of kynurenine metabolism emerged when in vitro studies on primary cultures of astrocytes and microglia stimulated by IFN-γ showed that kynurenic acid is produced mainly by astrocytes whereas quinolinic acid is only produced by microglia [40]. Since kynurenic acid acts as an antagonist whereas quinolinic acid acts as an agonist of NMDA receptors,

Müller, Myint, and Schwarz from the Department of Psychiatry at the University of Munich proposed that inflammation driven by Th1 cytokines such as IFN-γ shifts the equilibrium between these two opposite poles toward neurotoxicity by promoting microglial activation and downregulating astrocyte activity. Major depressive disorders would be the result of this shift. Conversely, Th2 cytokines such as IL-4 and IL-10 oppose the production and release of Th1 cytokines and therefore downregulate IDO. In association with astrocyte activation, this would result in an overproduction of kynurenic acid that would be responsible for schizophrenia [41].

A possible role for IDO activation in the pathophysiology of major depressive disorders was first hypothesized on the basis of the correlation between the fall in plasma levels of tryptophan and the intensity of depressive symptoms in cancer patients treated with IFN-α and/or IL-2 [3]. Preclinical studies carried out in mice injected with LPS or inoculated with BCG confirmed that pharmacological or genetic blockade of IDO activation abrogated the development of depression-like behavior without interfering with signs of sickness behavior [13,14]. These findings were interpreted to suggest that IDO activation functions as a molecular switch favoring the emergence of depression on a background of sickness [42]. However, further investigation into the metabolism of tryptophan in inflammation-induced depression could not reveal any evidence of tryptophan starvation in response to inflammation. Tryptophan levels in the brain remained constant or even increased in response to inflammation, despite the reduction in circulating tryptophan levels. Furthermore, detailed analysis of kynurenine metabolites based on tandem mass spectroscopy of the brains of LPS-treated mice revealed an activation of the kynurenine mono-oxygenase branch of kynurenine metabolism leading to 3-hydroxy kynurenic acid and quinolinic acid, without any change in the kynurenine aminotransferase branch leading to kynurenic acid [43]. These results essentially confirmed the hypothesis formulated by Müller and colleagues.

Because quinolinic acid acts as an agonist of the NMDA receptor, the next step was to test whether blockage of NMDA receptors abrogates inflammation-induced depression. Ketamine was selected for this purpose. However, ketamine has anti-inflammatory properties. It was therefore important to administer this compound at a time at which the inflammatory cascade triggered by inflammation had already developed and IDO was fully activated. This was done by administering ketamine immediately before the behavioral tests of depression-like behavior in mice treated 24 h earlier with LPS. As expected, this treatment abrogated the development of depression-like behavior in LPS-treated mice [43]. The confirmation that this effect was due to blockage of NMDA receptors rather than to another uncontrolled effect of ketamine was verified by injecting mice treated with LPS and ketamine with the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline-2,3-dione in order to block enhanced AMPA receptor glutamatergic neurotransmission due to NMDA receptor blockade. As expected, this treatment restored the LPS-induced depressive-like behavior that had been blocked by ketamine. Whether quinolinic acid alone is sufficient to induce depression-like behavior or whether it needs to

synergize with increased extracellular glutamate is discussed in the section on transport mechanisms below (see also [44]).

4 Transport Mechanisms Regulating the Communication Between Peripheral and Central Kynurenine Pathways

Transport mechanisms regulate the communication between peripheral and central kynurenine pathways under normal and inflammatory conditions [28]. Experiments carried out in pentobarbital-anesthetized rats using the in situ brain perfusion technique showed that kynurenine and 3-hydroxy kynurenine are transported into the brain along a concentration gradient by the large amino acid transporter LAT1, which also transports tryptophan and other branched-chain amino acids into the brain [45]. Among the other metabolites, only anthranilic acid enters the brain in sizeable quantities, but by passive diffusion. Although kynurenine can be formed in the brain by IDO present in microglial cells and brain macrophages, most of the brain kynurenine – 78% in gerbils – originates from the periphery [46]. In response to systemic immune activation caused by intraperitoneal injection of LPS, this percentage goes up to 100% despite the fact that IDO activity increases in the brain as well as in the periphery [6]. In this condition, brain quinolinic acid is formed mainly from blood kynurenine (52%) and blood quinolinic acid (40%), meaning that the permeability of the blood–brain barrier to kynurenine must increase [46]. When the inflammation is central rather than peripheral in origin, the brain production of kynurenine and quinolinic acid takes over and is responsible for more than 98% of the total brain levels of kynurenine and quinolinic acid [46].

LAT1 is a heterodimeric membrane transport protein that is composed of a heavy subunit protein 4F2hc/CD98 coded by the *SLC3A2* gene and a CD98 light subunit protein encoded by the *SLC7A5* gene. This sodium-independent transporter is abundant at the level of the blood–brain barrier, where it controls the influx of large neutral amino acids in the brain. It is also found in astrocytes, in which it probably controls the influx of kynurenine that is further metabolized into kynurenic acid [47]. Because LAT1 transports large neutral amino acids, such as leucine, phenylalanine, and tryptophan, it is theoretically possible to compete with the entry of kynurenine into the brain by increasing the concentrations of these competing amino acids at the periphery. Proof of principle for this possibility was recently obtained in vitro using cortical slices of rat brain incubated with kynurenine and competing amino acids for LAT1. Leucine and other branched-chain amino acids inhibited the uptake of kynurenine by brain slices [48].

Another transporter of importance for the relationship between inflammation and depression is system x_c^-. This system functions as an antiporter in the sense that it exchanges extracellular L-cystine for intracellular L-glutamate [49]. Like LAT1, x_c^- is a heterodimeric amino acid transporter composed of a heavy chain 4F2HC and a light chain xCT or SLC7A11. The intracellular transport of cysteine is

an essential step for the synthesis of glutathione, making system x_c^- an important player in intracellular redox processes. System x_c^- is upregulated in activated microglia, probably because of the necessity of these cells to protect themselves from the oxidative stress they are submitted to while producing inflammatory mediators. This is associated with an increased release of glutamate formed from glutamine via an enzymatic reaction catalyzed by glutaminase. LPS co-injected with cystine into the spinal cord gray matter induces glutamate-dependent neurotoxic inflammation [50]. Another mechanism by which inflammation can increase extracellular glutamate is by impairing astrocytic glutamate uptake [51]. In either case, excessive extracellular glutamate induces excitotoxicity by activating AMPA receptors. This effect could synergize with the NMDA-dependent effect of quinolinic acid to promote the development of depression.

5 IDO Activation and Behavioral Alterations in Other Animal Models of Inflammation

There is accumulating evidence of a role for IDO activation in the behavioral alterations that develop in animal models of exposure to various immune and non-immune insults.

Human immunodeficiency virus (HIV) infection is accompanied by a high rate of comorbid clinical depression. Intracerebroventricular administration of the HIV transactivator of transcription (Tat) protein induced depression-like behavior in mice, measured by increased immobility in the forced-swim test and decreased sucrose preference [52]. These behavioral alterations were associated with increased expression of IDO in the brain. Experiments with murine organotypic hippocampal slices confirmed that HIV Tat increased expression of IDO and showed that this effect was mediated by the p38 mitogen-activated protein kinase [53]. Viral infection can be mimicked by injection of polyinosinic:polycytidylic acid (poly I:C). Poly I:C is structurally similar to double-stranded RNA and differs from LPS by activating Toll-like receptor (TLR)3 instead of TLR4. Systemic administration of poly I:C to rats induced behavioral signs of anxiety and depression associated with increased expression of IDO in the frontal cortex and hippocampus up to 48 h after treatment [54]. Pneumococcal meningitis was associated with long-term changes in motor activity and cognitive deficits in recovered mice [55]. Motor activity was no longer altered in IDO knockout mice, but cognitive deficits were still present.

Autoimmune disorders are also frequently associated with clinical depression, but the role of IDO in this association has not been studied in a systematic manner. In a murine model of lupus, the development of depression-like behavior was associated with increased levels of kynurenine pathway metabolites, but the causality was not tested [56]. Similar effects were observed in response to CD40 ligand–CD40 immune activation, a murine model of autoimmune disorders.

Despite evidence for increased plasma and brain levels of kynurenine and its metabolites, chronic administration of a selective IDO inhibitor had no effect on sickness behavior or decreased saccharin drinking, in contrast to what was observed after blockade of TNF-α action [57].

The observation that systemic infection in the elderly is often associated with an increased frequency of behavioral and cognitive complications is in agreement with the hypothesis of a chronic low-grade inflammation in aged subjects, the so-called inflammaging condition [58]. Aged mice responded to LPS by an enhanced induction of peripheral and brain IDO and an increased duration of depression-like behavior [59]. The increase in brain IDO expression was present in microglia isolated from aged mice [60]. Similar findings were observed in mice inoculated with BCG [61].

Because of the frequent co-occurrence of chronic pain and depression, the possibility that these two conditions share a common biological mechanism represented by inflammation-induced IDO activation was investigated in a rat model of inflammatory arthritis induced by intra-articular injection of complete Freund adjuvant [62]. This treatment increased IDO enzymatic activity in the hippocampus via an IL-6-dependent mechanism. Administration of the IDO inhibitor 1-methyl-tryptophan at the periphery or in the hippocampus abrogated both mechanical allodynia and increased immobility time in the forced-swim test in arthritic rats. In the same manner, IDO1 knockout mice did not develop hyperalgesia and increased immobility in the forced-swim test in response to intra-articular injection of complete Freund adjuvant, in contrast to wild type mice. These results were interpreted to indicate that brain IDO activity regulates both chronic pain and depression. However, the generality of this interpretation is questionable, as we could not demonstrate any role for IDO activation in chronic pain in a mouse model of peripheral neuropathy induced by spared nerve injury, despite a clear role for IDO in the depression-like behavior displayed by mice in response to spared nerve injury. In this last experiment, IDO activation was only observed at the periphery, not in the brain, and the depression-like behavior was caused by the increased formation of quinolinic acid as a result of the activation of the kynurenine mono-oxygenase branch of kynurenine metabolism in the hippocampus contralateral to the site of nerve injury [63].

Exposure to various stressors can activate the kynurenine metabolism pathway. However, whether this is due to immune-dependent IDO activation or to corticosterone-induced TDO activation is not always clear. Mice exposed to a model of unpredictable chronic mild stress to induce depressive-like behavior showed increased kynurenine metabolism both at the periphery and in the brain [64]. Maternal separation, which induces long-lasting behavioral alterations in mice, had the same effect [65]. Inescapable exposure of mice to a rat predator had also long-lasting effects on behavior and kynurenine metabolism in the brain [66]. The role of TDO was assessed in the development of increased immobility in the forced-swim test in rats submitted to chronic restraint for 2 h per day. Inhibition of TDO by allopurinol abrogated the chronic stress-related increase in immobility and the accompanying increase in circulating kynurenine levels [67].

Various forms of brain injury can activate IDO. Cerebral ischemia–reperfusion in mice enhanced IDO activity as measured by increases in the plasma ratio of kynurenine to tryptophan and IDO expression in cerebral arterioles [68]. However, blockade of IDO activation by 1-methyl-tryptophan or genetic deletion of *ido1* did not affect overall outcomes as measured by neurological function and total brain infarct volume and swelling. Whether IDO activation plays a role in the etiology of post-stroke depression has not yet been tested [69]. In a rat model of chronic temporal lobe epilepsy induced by a combination of lithium chloride and pilocarpine, blockade of IDO activation by the anti-inflammatory tetracycline derivative minocycline or the IDO antagonist 1-methyl-tryptophan abrogated epilepsy-associated depression-like behavior measured by decreased sucrose preference and increased immobility in the forced-swim test [70]. However, blockade of IDO activation had no effect on spontaneous seizures.

6 Targeting Inflammation-Induced Depression: Translational Aspects

The demonstration of a causal relationship between inflammation-induced activation of IDO and the kynurenine metabolism pathway, on one hand, and depression, on the other hand, opens a number of opportunities for treatment of inflammation-induced depression. Figure 5 represents the different steps of the process leading from inflammation to depression. Administration of anti-inflammatory drugs represents an obvious option. However, all of the attempts to treat depression by targeting inflammation have been made by administering the anti-inflammatory compound preventively. Several instances of this type of study have been mentioned in the previous sections of this chapter. In general, minocycline and cytokine antagonists are effective to block inflammation-induced depression when administered before immune stimulation. The same effect is obtained with a wide variety of anti-inflammatory or anti-oxidant natural compounds, such as curcumin, apigenin, honokiol, xiaobuxin-tang flavonoid extract, perillaldehyde, ginseng saponins, and alpha-tocopherol [71–77]. However, it is not known whether the anti-inflammatory treatment can block behavioral signs of depression once they have developed.

Classical antidepressants have been shown to have anti-inflammatory properties that vary depending on the drug, the treatment schedule, and the type of assay [78]. An optimum strategy to treat inflammation-associated depression would then be to select the antidepressant that combines anti-inflammatory and antidepressant properties. However, it is difficult to classify antidepressants on the basis of their anti-inflammatory properties because the data that are available in the literature are often contradictory. In addition, there has been no attempt to relate at the preclinical level their anti-inflammatory activity with their ability to decrease depression-like behavior. Most studies have been carried out with the specific serotonin reuptake inhibitor fluoxetine. Fluoxetine was able to abrogate depression-like behavior

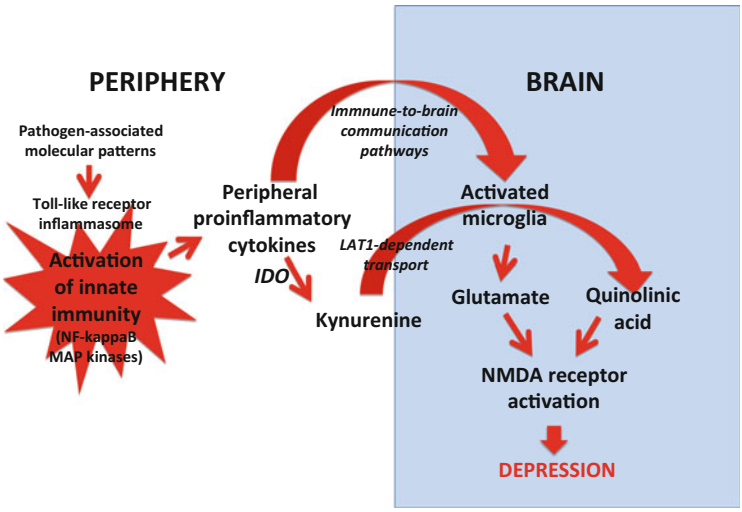

Fig. 5 Pathophysiology of inflammation-induced depression. Activation of innate immunity by binding of pathogen-associated molecular patterns (e.g., lipopolysaccharide) to Toll-like receptors and inflammasome elements induce the production and release of proinflammatory cytokines at the periphery, which in turn recruit immune-to-brain communication pathways and activate microglia. Activated microglia produce and release proinflammatory cytokines that organize the sickness response to pathogen-associated molecular patterns (not represented in the figure). Activation of indoleamine 2,3 dioxygenase at the periphery increases kynurenine that is transported into the brain by LAT1 and metabolized into neurotoxic kynurenine metabolites (e.g., quinolinic acid) at the level of activated microglia via a series of enzymatic reactions initiated by mitochondrial kynurenine mono-oxygenase. Quinolinic acid alone or in combination with extracellular glutamate released by activated microglia activates *N*-methyl-D-aspartate receptors

induced by the parasite *Trypanozoma cruzi* in mice [79]. Although the parasitic disease was associated with IDO activation, there was no indication that fluoxetine acted by blocking IDO activation. Fluoxetine was also able to abrogate depression-like behavior induced by systemic TNF-α in mice, but once more there was no indication that this was due to the anti-inflammatory effect, if any, of fluoxetine [80]. The only study in which this aspect was considered yielded negative results: chronic administration of fluoxetine blocked the increased immobility displayed by tumor-bearing mice in the forced-swim test, but this effect was not associated with any alteration in expression of hippocampal proinflammatory cytokines and kynurenine mono-oxygenase [81]. In contrast, ibuprofen attenuated both depression-like behavior and hippocampal proinflammatory cytokine expression [82]. It is important to note that all forms of inflammation-induced depression are not sensitive to fluoxetine treatment. BCG-induced depression-like behavior was actually resistant to acute administration of fluoxetine and escitalopram but sensitive to acute administration of the tricyclic antidepressant imipramine, the dual serotonin/norepinephrine reuptake inhibitor duloxetine, and the dual dopamine/norepinephrine reuptake inhibitor nomifensine [83].

IDO activation is the molecular switch that favors the transition from sickness to depression; thus, it should be possible to treat inflammation-induced depression by blocking IDO. Although this works well in experimental studies of inflammation-induced depression, there are several obstacles to this strategy, represented by the lack of approved IDO antagonists, the possible side effects of IDO antagonists on the immune system and in particular the increased risk of autoimmune disorders, and the lack of data on the ability of IDO antagonism to reverse signs of depression when used for cure rather than for prevention.

Because most of the kynurenine that is found in the brain during systemic inflammation comes from the periphery, it should be possible to target the transport mechanisms that control kynurenine influx into the brain to prevent the formation of neurotoxic kynurenine metabolites. The administration of branched-chain amino acids competing with kynurenine for transport via LAT1 represents a theoretically viable solution, providing this does not at the same time limit the brain influx of tryptophan. L-leucine is a good candidate for this strategy because it simultaneously upregulates LAT1, decreases the production of proinflammatory cytokines, and activates mTOR in the brain, which could favor synaptic plasticity [84–86]. We have obtained encouraging preliminary results in LPS-treated mice administered L-leucine. Systemic administration of this amino acid before and 6 h after LPS abrogated depression-like behavior measured 24 after LPS. This effect was associated with a decrease in brain kynurenine levels but no change in brain tryptophan levels [87].

Other ways of interfering with the formation of neurotoxic kynurenine metabolites include blocking kynurenine mono-oxygenase or favoring the kynurenic acid branch of kynurenine metabolism. Inhibition of kynurenine mono-oxygenase blocked the depression-like behavior developed by mice submitted to spared nerve injury [63]. In the same manner, both kynurenine mono-oxygenase-deficient mice and IDO-deficient mice were protected from inflammation-induced deficits in novel-object recognition [88]. Kynurenine mono-oxygenase inhibition not only inhibits the formation of 3-hydroxy kynurenine and quinolinic acid, but also increases the brain concentrations of the neuroprotective metabolite kynurenic acid. This strategy has therefore been proposed to ameliorate the neurodegeneration that develops in a mouse model of Huntington disease [89]. However, the specificity of the drug used to block kynurenine mono-oxygenase has been questioned [90].

Convergence of neurotoxic kynurenine metabolites and excess extracellular glutamate on glutamatergic neurotransmission makes NMDA receptor a potential target for treatment of inflammation-associated depression. As mentioned earlier in this chapter, preventive or curative administration of the noncompetitive NMDA receptor antagonist ketamine abrogated LPS-induced inflammation [43]. Ketamine has some anti-inflammatory properties [91,92], but they did not account for the antidepressant effect observed in LPS-treated mice [43]. The clinical use of ketamine is limited by its psychotomimetic properties. The current development of NMDA receptor antagonists lacking these negative side effects will certainly

provide new antidepressant compounds, the ability of which to treat inflammation-associated depression will still have to be determined.

7 Conclusion

Diagnostic criteria for major depressive disorder and depressive episodes carefully eliminate depressed mood associated with medical illness. However, depressed patients are rarely free of other medical complications. Psychiatrists commonly encounter in their clinical practice patients with major depressive disorders who have chronic coexistent medical conditions, including chronic inflammation disorders. Studies of the relationship between inflammation and depression have shed some light on the reasons for this comorbidity. Depression does not emerge out of nothingness. In the same way that anxiety disorders derive from alterations in the fear motivational system involved in the processing of real and potential threats and the organization of subjective, behavioral, and physiological responses to these threats, depressive disorders emerge from alterations in the sickness motivational system that is responsible for reorganizing priorities in an organism at the juncture of life and death because of an ongoing infectious process. It has been possible to understand how sickness transitions into depression because the preclinical investigations at the origin of this research have carefully built on what has been learned in the clinic, in inflamed patients who develop clinical symptoms of depression. Psychobiologists have walked hand in hand with clinical psychologists and psychiatrists to design animal models of inflammation-induced depression and elaborate testable hypotheses on its pathophysiology. This research has resulted in the appearance of a number of new players on the stage of biological psychiatry, from inflammatory molecules to transport mechanisms regulating the communication between peripheral and central kynurenine metabolites.

It is possible that this adventure into inflammation-associated depression will mainstream research in biological psychiatry on a final common pathway for both immune and non-immune factors in depression, represented, for instance, by alterations in NMDA receptor activation. Even if this is the case, the inflammation detour will not have been vain since it will have allowed researchers to demonstrate unequivocally that depression is not just a disease of the neuron. It is now clear that depression is a disease of the communication between endothelial cells, glia, and neurons, and this communication is profoundly dependent on systemic factors, including inflammation.

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Depression in Autoimmune Diseases

Christopher R. Pryce and Adriano Fontana

Abstract Up to 50% of patients with autoimmune diseases show an impairment of health-related quality of life and exhibit depression-like symptoms. The immune system not only leads to inflammation in affected organs, but also mediates behavior abnormalities including fatigue and depression-like symptoms. This review focuses on the different pathways involved in the communication of the immune system with the neuronal network and the body's timing system. The latter is built up by a hierarchically organized expression of clock genes. As discussed here, the activation of the immune system interferes with high amplitude expression of clock genes, an effect which may play a pivotal role in depression-like behavior in autoimmune diseases.

Keywords Hepatitis • Indoleamine-2,3-dioxygenase • Interleukin-1 • Kynurenine • Rheumatoid arthritis • Systemic lupus erythematosus • Tumor necrosis factor • Twist1

Contents

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1 Introduction

In autoimmune diseases, psychological symptoms, such as fatigue, loss of interest in daily activities, and cognitive deficits, are common. Whilst these symptoms are often described as sickness behavior, they are also core symptoms in the diagnosis of depression. This raises a number of issues, and perhaps most important is the issue of whether sickness behavior and depression are one and the same, or do they have fundamentally different pathophysiologies so that it is more appropriate to regard them as separate clinical states? The present chapter reviews the evidence for inflammation in depression and for depression-like symptoms in autoimmune diseases. It is not currently possible to unequivocally answer the issues raised above. However, we do provide an overview of some of the most relevant evidence that can inform this controversial, interesting, and medically important debate.

Patients with depression, most notably major depressive disorder (MDD), who are otherwise medically healthy have been found to exhibit features of immune activation. Increases of the circulating cytokines interleukin (IL)-6 and tumor necrosis factor alpha (TNF), their soluble receptors, and the acute phase protein, C-reactive protein (CRP), have been reported [1]; for review, see [2, 3]. Moreover, gene expression profiling conducted on post-mortem brain tissue samples from the frontal cortex of patients with MDD shows an upregulated expression of cytokines including, e.g., IL-1β, IL-18, IL-8, IL-12, lymphotoxin alpha, and interferon (IFN)-γ [4]. More direct evidence for the involvement of cytokines in the pathophysiology of MDD comes from the observations that (1) anti-TNF treatment has therapeutic effects in resistant MDD with increased CRP [5], and (2) the development of depressed mood, anxious symptoms, and impaired cognition in human patients treated with type I interferons [3]. Likewise, in mice, the systemic or intracerebroventricular administration of cytokines or cytokine inducers leads to depressive-like behavior [6]. Pathways that translate the cytokine signals from the periphery to the brain involve: (1) actions of cytokines on the vagal nerve and activation of Toll-like receptors in circumventricular organs, (2) interactions of cytokines with the hypothalamic–pituitary–adrenal axis, and (3) cytokine triggered activation of indoleamine-2,3-dioxygenase leading to tryptophan catabolism and decreased serotonin concentrations in the central nervous system (CNS) (for review see [7, 8]).

In light of the putative causal link of increased cytokines and depression, we may ask whether depression-like symptoms or depression per se is also a hallmark in

autoimmune diseases. The latter are characterized by anti-self T- and B-cell reactivity, which leads to organ dysfunction due to destruction of parenchymal cells in various organs, including kidney, lung, liver, intestinal tract, and the peripheral and central nervous systems. The immune response towards autoantigens is characterized by activation of neutrophils, monocytes–macrophages, dendritic cells, and lymphocytes on the one hand, and decreased activity of negative feedback loops provided by regulatory T- and B-cells and by suppressive macrophages, on the other. Both the priming and effector phases of the autoimmune response are associated with increased production of cytokines. Since inducers of cytokines and cytokines per se induce depressive-like behavior in both experimental animal models and humans it is not surprising that autoimmune diseases have also been associated with depression. In the following we will focus on the evidence for depression-like symptoms in autoimmune diseases and animal models thereof. We will concentrate on systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), since in these diseases large cohort studies on mental health have been conducted and therefore a relatively large amount of data are available. However, depression-like symptoms have also been well described in many other autoimmune diseases including multiple sclerosis and inflammatory bowel disease (for review see [9, 10]). Moreover, depression-like symptoms are also a hallmark of autoinflammatory diseases. These inherited diseases are characterized by inadequate activation of the immune system with production of cytokines due to mutations in genes encoding for the inflammasome. The most frequent form is familial Mediterranean fever (FMF), which develops due to mutations of the pyrin/FMF gene. In FMF patients depression-like behavior is seen frequently; the depression-like symptom severity does not depend on FMF duration, but rather on the FMF severity score [11].

2 Depression-Like Symptoms in Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by loss of tolerance, which is associated with production of autoantibodies and immune mediated injury resulting in tissue damage in various organs including kidney, lung, skin, and central nervous system. Activation of the complement system by immune complexes leads to endothelial cell disruption. SLE takes a relapsing-remitting disease course which, depending on the affected organ, can be mild or severe. Numerous studies show that patients with SLE have a significantly poorer health-related quality of life (HRQoL) and exhibit depression-like symptoms more often than do healthy controls. In a systematic literature search 17 studies were identified that reported on depression comorbidity symptoms, with a prevalence rate of 17–75% depending on study [12]. Different methods were used to

assess depression across these studies, and this is likely to contribute to the wide range in the prevalence reported.

A study on 170 SLE patients found HRQoL to be negatively influenced mainly by disease activity and depression [13]. Both parameters were associated with higher levels of pain and fatigue [14, 15]. Using the Beck Depression Inventory (BDI), patients with SLE had a higher score compared with the general population from a pan-Europe population based study [16]. The prevalence of depression was found to be 16.6% in SLE and 6.7% in controls. There was no association between disease activity and organ damage. Likewise in 113 patients with SLE no relationship between HRQoL and SLE activity or disease duration was observed [17]. In 110 lupus patients the SLE activity index did correlate with the anxiety score, but not with depression [18]. Another study using BDI-II showed 53 of 127 SLE patients (41.7%) to have moderate or severe depressive symptoms [19]. When assessing sleep behavior in 81 patients with SLE, depressive symptoms were associated significantly with poor sleep quality, and cognitive deficits occurred in 16 of 82 patients [20]. Impaired cognitive performance in sustained attention and spatial working memory tests was related to SLE, but not to depression [21]. With respect to the important question of whether depression-like symptoms in autoimmune-disease patients results in poorer disease control, in general it is indeed thought that depression is associated with increased disease activity, decreased medication adherence, and decreased work productivity [22, 23].

Approximately 15–20% of SLE cases begin in childhood, at a median age of onset of 12–13 years. In children and adolescents with SLE ($n = 38$) and young adults with childhood-onset of disease ($n = 16$), depression was observed in 26 and 44% of the patients, respectively, [24]. Depression was not correlated with disease duration, HRQoL, or SLE nephritis. In pediatric lupus and mixed connective tissue disease, depression was associated with poor disease control; non-Caucasian patients were at significantly higher risk for depression-like symptoms than Caucasian patients [25, 26]. In general disease scores, lupus nephritis and involvement of the central nervous system—neuropsychiatric lupus (NPSLE)—take a more aggressive course in African-Americans. In this group of patients, higher perceived racism was associated with having moderate to severe depression [27]. Suicidal ideation has been observed in pediatric lupus with NPSLE in 34% of 53 patients, and 20% of those with ideation attempted suicide. In adults with SLE, suicidal ideation is common in up to one-third of patients with and without NPSLE, and is associated with higher disease activity, increased depression and anxiety severity, and previous suicide attempts [28–30].

The mechanisms underlying depression or depression-like symptoms in SLE are not yet clear, but are likely to be multifactorial. The burden of having a chronic illness, the use of corticosteroids and immunosuppressive drugs for disease control and prevention of disease, microvasculopathy as well as the development of secondary cerebrovascular injury due to hypertension, hyperlipidemia, and antiphospholipid antibodies, may all constitute risk factors in SLE. Depression in women with SLE ($n = 161$) correlates with the presence of cardiovascular disease [31]. Serum TNF was found to be higher in SLE ($n = 54$) compared to controls.

Interestingly, increased TNF was associated with more severe depression-like symptoms as evaluated by the Hospital Anxiety and Depressive Scale (HADS) [32]. Injection of TNF in rodents leads to sickness behavior and depression-like behavior [6]. Patients with SLE exhibit high serum levels of IFN type I and overexpress IFN-I-stimulated genes in peripheral blood cells. The increased IFN-I signature is associated with active disease including lupus nephritis. Since in patients with multiple sclerosis or hepatitis C, treatment with IFN-α induces depression and the risk of suicidal ideation, the endogenous expression of IFN-I in SLE may contribute to depression-like symptoms. However, in patients with SLE the increased IFN-I levels are found to not be correlated with depression and fatigue [33].

Polymorphisms of the serotonin transporter gene promoter region (PR-5HTT) were studied in 96 SLE patients. Using the Hamilton depression rating scale (HDRS), depression scores were found to be associated with patients being homozygous for the short allele of the PR-5HTT gene [34]. The methylation status of the region studied did not differ in patients with depression versus those without. In neuropsychiatric lupus, anti-ribosomal P antibodies (Ab) have been reported to occur more frequently in SLE patients with depression [35]. In patients with a disease duration of less than 2 years, depression severity was correlated with anti-ribosomal P Ab levels. The antibody recognizes the carboxy 22 amino acids of the three large subunit ribosomal phosphoproteins, called P0, P1, and P2. Anti-ribosomal P Ab bind to neurons in the hippocampus, cingulate cortex, and the primary olfactory piriform cortex, and cause calcium influx and apoptosis [36]. Intracerebroventricular injection of anti-ribosomal P Ab in mice induces a depression-like behavior and olfactory impairment. Mice displayed impaired passive avoidance [37]. In the pathophysiology of NPSLE, anti-double stranded (ds)-DNA Ab may contribute to the involvement of the central nervous system. Some anti-native-DNA Ab cross-react with the GluN2A and GluN2B subunits of the *N*-methyl-D-aspartate (NMDA) receptor for the neurotransmitter glutamate [38]. Based on mouse experiments, cross-reacting antibodies may contribute to the neurocognitive impairment and other symptoms of NPSLE. When injected with the 16/6 idiotypic antibodies, which recognize anti-ds-DNA Ab as well as brain glycoproteins and glycolipids, mice develop an impairment of spatial memory [39, 40].

3 Rheumatoid Arthritis and Depression-Like Symptoms

In addition to its symptoms in affected joints, rheumatoid arthritis (RA) is associated with substantial mental health problems. In particular, depression has been documented to contribute significantly to impaired HRQoL, unemployment, and loss of work productivity. Depression is very common in RA, the prevalence reported to be 13–42% [41]. As reviewed recently, depression in RA provides an independent risk factor for cardiovascular disease and myocardial infarction,

suicidal ideation, and death, even after controlling for RA disease duration, disease activity, disability, and pain [42]. Depression in RA patients is most common in females and in younger age groups [43]. The risk to develop depression is highest in the first 5 years after RA diagnosis [44]. Whilst pain is likely to contribute to the development of depression, the link between the severity of measurable parameters of arthritis and depression is less stringent. Since cytokines play a pivotal role in the synovial inflammation in RA and TNF blockers exert strong therapeutic effects, it may be suggested that the cytokine–brain connection is essential in depression in RA. The hypothesis is supported by the observation of persistence of depression in patients with poor disease control by TNF blockers [45]. However, studies investigating an association between depression in RA and increased CRP have yielded conflicting findings [42]. In a recent study on RA ($n = 102$ with 75% females), using the Beck depression index-II (BDI-II) to assess depression, and the Health assessment questionnaire (HAQ) and the Disease activity score (DAS) to quantitate RA activity, a correlation of severity of depression and RA activity was identified [46]. Somewhat in line with these data is the finding that functional disability constitutes a significant risk factor for the occurrence of depression in RA [47]. Amelioration of depression symptoms in RA is linked to treatment-related RA control, positive psychological coping mechanisms, and social support (for review see [42]).

4 Depression-Like Behavior in Animal Models of Autoimmune Diseases

It is well established that rodents injected systemically or intracerebroventricularly with cytokines including TNF and IL-1β show an increase in sleep at times when mice are usually active (for review see [48, 49]). NREM sleep was found to be increased in the dark phase of the 12 h light–dark circadian cycle. This effect is associated with increase in body temperature, loss of body weight, and development of depression-like behavioral changes. The same clinical picture emerges when mice are treated with activators of Toll-like receptors including the synthetic analogs of ds-RNA, namely Poly(I:C), lipopolysaccharide (LPS) extracted from gram-negative bacteria, or peptidoglycans. These experimental animal models present with depression-like behavior due to activation of the innate immune system. Animal models of depression-like behavior that are based on activation of the acquired immune system are less well established. In the following sections we discuss two models which do indeed involve or mimic effects of the aforementioned acquired immune mediated pathway (Fig. 1).

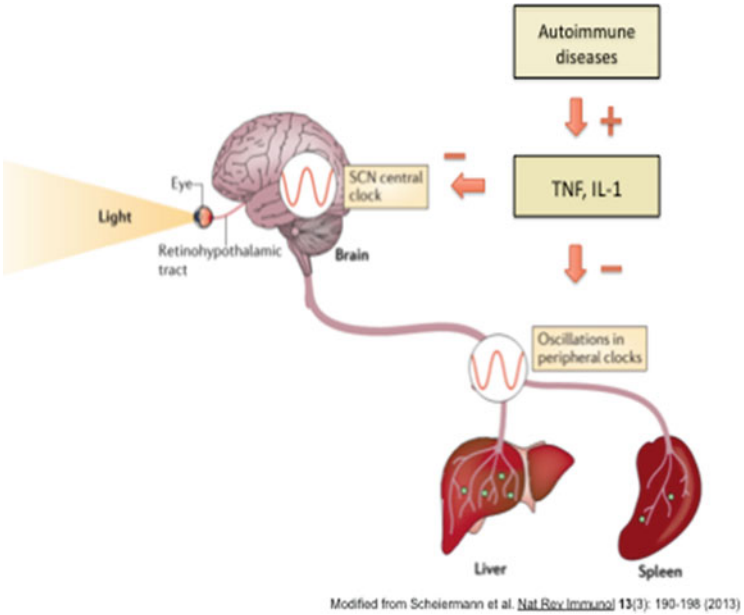

Fig. 1 Cytokines and the clock. Alterations of the circadian system may play a pivotal role in depressive-like behavior. Cytokines interfere with the physiologic circadian oscillation of clock genes in the suprachiasmatic nucleus of the brain, which receives signals via the light–dark cycle. The oscillation of the peripheral clock is altered in autoimmune diseases in various organs including the liver and colon in hepatitis and colitis, respectively. This may cause abnormal expression of circadian genes. The clock system is also abnormal in lymphoid organs including the spleen and bone marrow. Thereby immune mediated effector pathways may be impaired

4.1 Depression-Like Behavior in MRL/lpr Mice

MRL lymphoproliferation (MRL/lpr) strain mice develop a systemic autoimmune disease with arthritis, nephritis, and neurological-psychiatric disease similar to SLE in humans. The mice show progressive lymphadenopathy due to the accumulation of double negative $CD4^{-}8^{-}B220^{+}$ alpha $beta^{+}$ T cells. The underlying defect involves the expression of a functionally inactive Fas receptor, which physiologically promotes activation induced cell death. Prior to the stage at which autoantibodies against DNA and nucleoproteins become detectable, MRL/lpr mice develop depression-like behavior including reduced activity in the forced swim-test and reduced preference for sweet solutions in the two-bottle sweet versus water test (for review see [50]). Thus studies on the pathophysiology of depression-like disease in the MRL/lpr autoimmune mouse offers potential new insights into the understanding of depression-like symptoms in SLE patients.

4.2 Activation of CD40 Leads to Depression-Like Behavior in Mice

To study the etio-pathophysiology of depression-like behavior in autoimmune diseases, we have developed a new mouse model, which is based on immune activation by CD40L–CD40 interactions. The CD40 ligand (CD40L)–CD40 pathway is essential for the autoimmune response to self-antigens [51]. Mice with neutralization of this ligand-receptor pathway are protected from experimental autoimmune diseases such as experimental autoimmune encephalitis (EAE). Moreover patients with depression have increased plasma levels of soluble CD40 and CD40L [52, 53]. CD40L is expressed mainly by activated CD4+ T cells and binds to CD40 expressed on macrophages, B-lymphocytes, and dendritic cells. CD40 activation by agonistic anti-CD40 monoclonal antibodies (mAb) induces synthesis of chemokines and cytokines including TNF, IL-18, and IFN type I and II. Immune activation is associated with multiorgan inflammation with severe necrotizing hepatitis, lymphadenopathy, splenomegaly, and depression-like behavior in the form of weight loss, decreased feeding, decreased activity, and increased NREM sleep [51, 54, 55]. These depression-like behaviors persist up to day 3. Furthermore, CD40 Ab also led to decreased operant motivation for and consumption of sweet-tasting saccharin solution up to day 7 and decreased fear conditioning of a tone to electroshock at day 5, indicating induction of reduced interest in reward and impaired learning/plasticity, respectively [54]. These behavioral effects were coincident with increases in TNF as well as kynurenine and its downstream catabolites in plasma and brain up to days 7–8. Co-injection of the TNF blocker etanercept with CD40 Ab prevented each of weight loss, decreased activity, increased sleep, and reduced interest in reward, and markedly attenuated kynurenine pathway activation in plasma and brain. However, co-injection of an inhibitor of indoleamine-2,3-dioxygenase, the enzyme primarily responsible for tryptophan-to-kynurenine conversion, was without effect on either weight loss or saccharin drinking [54]. According to this mouse model, activation of TNF is necessary for causation of depression-like behavior in autoimmune disease. Extrapolating the findings to human suggests that symptoms such as decreased appetite, loss of interest-pleasure, and cognitive impairments that are highly comorbid with autoimmune disorder have an etio-pathophysiology that begins with CD40–CD40L-TNF activation, whilst downstream factors remain to be elucidated.

5 Abnormal Clock Gene System in Autoimmune Diseases and Depression

Circadian rhythms are mediated by clock genes, and thereby regulate metabolism and sleep–wake behavior [56, 57]. The main transcription factors coordinating the circadian rhythms are CLOCK and BMAL1, which form heterodimers and activate

expression of *Period* (*Per*), *Cryptochrome* (*Cry*), and of various clock controlled genes by binding to E-box motives [58] (Fig. 2). Furthermore, CLOCK can be substituted by its paralog NPAS2 [59]. The binding of CLOCK:BMAL1 to the E-box is regulated by positive and negative feedback mechanisms [58]. Patients with depression show sleep disturbances and an abnormal circadian regulation of hormones including cortisol and melatonin [60]. Abnormal rhythms of expression of clock genes, due to effects of cytokines on transcription and posttranslational processes including chromatin remodelling, may play a role in depression. Increased risk for developing depression has been identified in individuals with polymorphisms carrying the CC genotype in Cry1 rs2287161 and the TT genotype in Tef rs738499 [61]. An analysis of post-mortem brain tissues from 55 normal controls and 34 patients with depression shows, in the patients, an abnormal phasing of circadian gene expression and potentially disrupted phase relationships between individual circadian genes [62]. Also, the cyclic patterns were much weaker in the depression patients.

Recent studies point to communication of the body clock and the immune system. For example, the macrophage response to *Salmonella* is decreased in mice with an inactive *clock* gene [63], and the extent of secretion of TNF and IL-6 by LPS stimulated macrophages follows a circadian rhythm [64]. Besides effects of the circadian system on the immune response, the immune system also influences the circadian clock. TNF inhibits the expression of all three *Period* genes, of *Cry-1* and *-2*, and of the PAR-bZip transcription factors *Dbp, Tef*, and *Hlf* [65, 66]. At least some of these effects are due to an interference with E-box-dependent transcription [65]. Both TNF induced Twist1 expression and TNF-mediated inhibition of the cold-inducible RNA binding protein (CIRBP) are involved in immune mediated dysregulation of clock genes [67, 68]. In light of the abnormal clock gene expression in depression, the polymorphisms in the Cry-1 and Tef genes associated with depression, and the dysregulated expression of clock genes in cells exposed to TNF and IL-1β, we hypothesize that depression in autoimmune diseases may involve cytokine induced dysregulation of expression of clock genes. The hypothesis is supported by recent findings of abnormal expression of clock genes in rheumatoid arthritis, known for its high depression comorbidity (see above). When arthritis is induced in C57BL/6 mice by i.p. injection of anti-type II collagen mAb on day 1 and LPS (50 μg) on day 2, the expression of Bmal1, Per2, and Dbp was decreased in the 24 h cycle in spleen and the joints [69]. When analyzing clock genes in cultured human rheumatoid synovial cells treated with TNF, suppression of the expression of *Per2, Dbp, Tef*, and *Hlf* was found [70]. Recently, a loss of circadian rhythmicity in the expression of the clock genes *Per2* and *Per3* in CD14+ monocytes was identified in postmenopausal patients with rheumatoid arthritis [71]. A down regulation of the aforementioned genes was also observed in a transcriptome analysis of genes expressed in the colon mucosa of patients with inflammatory bowel disease [72]. The topic of clock genes, depression, and autoimmune diseases has just started to become interesting. From first studies we conclude that the circadian expression of clock genes in

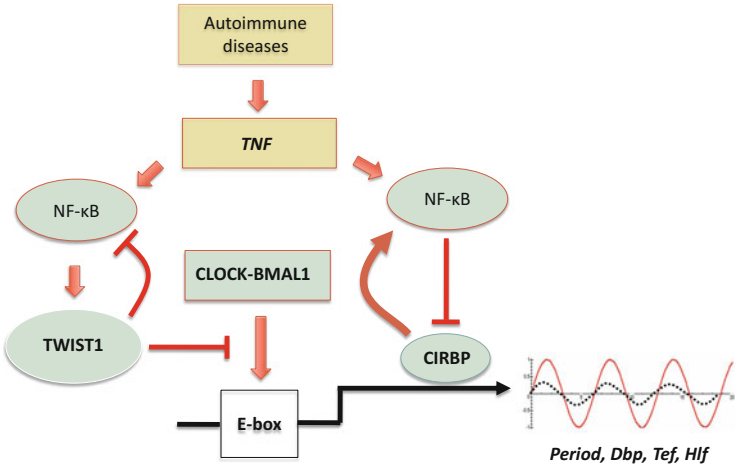

Fig. 2 Impaired expression of clock genes due to effects of proinflammatory cytokines. TNF and IL-1b decrease high amplitude expression of clock genes by inhibiting transcription of the cold-inducible RNA binding protein (CIRBP). Inhibition of CIRBP expression involves NF-kB activation. CIRBP enhances NF-kB expression and thereby, e.g., regulates TNF-mediated apoptosis. In addition to CIRBP, Twist1 is also involved in altering clock gene expression. TNF and IL-1b induce Twist1, which interferes with the activation of E-box mediated transcription by CLOCK-BMAL1. This effect leads to impaired expression of the E-Box regulated clock genes Period-1, -2, and -3, the cryptochrome genes Cry-1 and -2, and the PAR-bZip transcription factors Dbp, Tef, and Hlf. However, recent studies on the expression of clock genes in colon and inflammatory monocytes of mice with DSS induced colitis do not support the in vitro findings of Twist1 to be involved in suppression of clock genes (Seles C., Strauss L, Rambousek L., and Fontana A., manuscript in preparation)

autoimmune diseases is altered. It remains to be seen whether the defect is associated with behavioral abnormalities and/or abnormal immune regulation (Figs. 2 and 3).

6 Concluding Remarks

The link between immune activation and depression-like behavior becomes evident from studies on injection of cytokines and their triggers such as LPS, PolyI:C, and CD40 mAb in rodents. Autoimmune animal models, e.g., MRL/lpr mice or mice treated with agonistic CD40 mAb, support the hypothesis that immune effector pathways cause depression-like behavior. From the studies in SLE and RA outlined above, it can be concluded that in addition to organ specific symptoms arising due to autoimmune inflammation, depression or depression-like symptoms are also a severe complication, which impairs quality of life. The reports on associations of immune activation with depression-like symptoms point to multiple underlying pathways impacting on these psychological states. Cytokines, autoantibodies, and

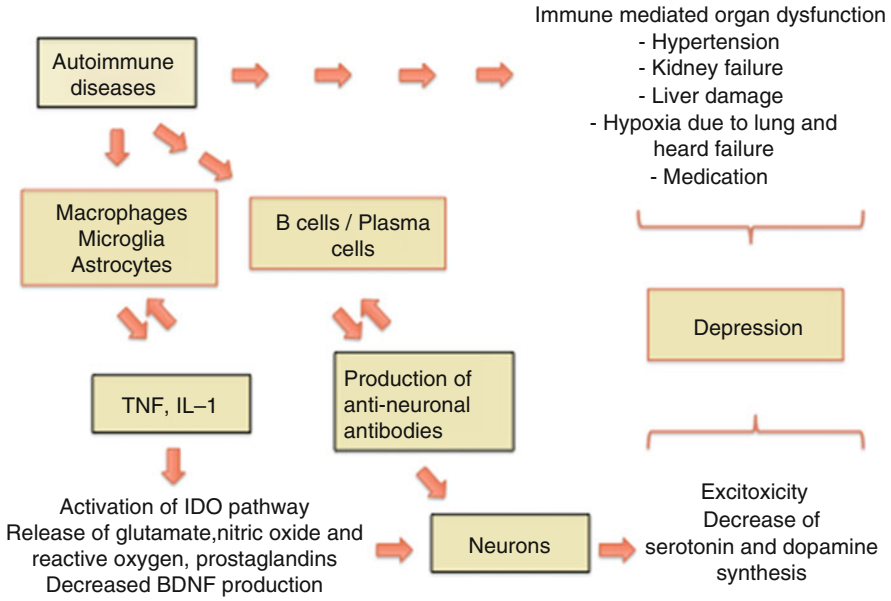

Fig. 3 Multiple pathways may lead to depression-like behavior in autoimmune diseases. Immune mediated tissue damage may lead to depression due to the development of hypoxia in heart and pulmonary failure, or due to the development of hypertension in kidney diseases. Depression is also caused by medication used to treat autoimmune diseases, such as treatment with interferon type 1 in multiple sclerosis or corticosteroids in systemic lupus erythematosus. Recognition of self-antigens leads to activation of microglia and macrophages, and of astrocytes, which produce cytokines acting in an autocrine and paracrine manner. This step is followed by the release of molecules including excitatory amino acids, nitric oxide, and reactive oxygen species, which alter synaptic signalling pathways and, e.g., cause NMDA mediated synaptic activation. The latter effect is enhanced by impaired astrocyte glutamate detoxification. Neurotoxicity is amplified by oxidative stress and toxic effects of TNF on one hand, and poor neurotrophic support on the other. Autoantibodies to NMDA receptors and ribosomal P protein as detected in SLE may contribute to neuronal damage

complement factors have all been shown to alter neuronal activity, either directly, by modulating the production of glutamate by microglia, or, in the case of TNF, by enhancing neuronal activity via activation of TNF receptors on astrocytes [73, 74]. When taking one distinct immune effector pathway such as proinflammatory cytokines, their involvement in depression-like symptoms may not be clearly evident because other mechanisms, e.g., in SLE, anti-NMDA receptor antibodies, or anti-ribosomal P antibodies, may co-occur and, in contrast to cytokines, the production of autoantibodies may continue into the remission phase. In addition, the consequences of effects of cytokines and autoantibodies on brain circuitries may perpetuate beyond the active disease phase and cause persistence of depression-like symptoms in an autonomic fashion in the absence of the triggering immune effector pathways. In addition to immunological mechanisms, many other variables including organ dysfunction (e.g., nephritis and hypertension in SLE),

treatment including IFN type I and corticosteroids, social factors, functional disability, and coping strategies may be confounding in studies aimed at demonstrating a (causal) relationship between immune activation and depression-like symptoms. Whilst it remains to be determined to what extent psychosocial-stress induced depression symptoms and depression-like symptoms in autoimmune diseases have common or separate pathophysiologies, it is clear that mood, motivation, fatigue, and cognition are frequently disrupted in autoimmune disorders, constitute a major component of the disease, and need to be the focus of research efforts designed to improve their treatment.

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Stress-Induced Microglia Activation and Monocyte Trafficking to the Brain Underlie the Development of Anxiety and Depression

Karol Ramirez, Jaime Fornaguera-Trías, and John F. Sheridan

Abstract Psychosocial stress is capable of causing immune dysregulation and increased neuroinflammatory signaling by repeated activation of the neuroendocrine and autonomic systems that may contribute to the development of anxiety and depression. The stress model of repeated social defeat (RSD) recapitulates many of the stress-driven alterations in the neuroimmune system seen in humans experiencing repeated forms of stress and associated affective disorders. For example, RSD-induced neuronal and microglia activation corresponds with sympathetic outflow to the peripheral immune system and increased ability of bone marrow derived myeloid progenitor cells (MPC) to redistribute throughout the body, including to the central nervous system (CNS), reinforcing stress-associated behaviors. An overview of the neuroendocrine, immunological, and behavioral stress-induced responses will be reviewed in this chapter using RSD to illustrate the

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mechanisms leading to stress-related alterations in inflammation in both the periphery and CNS, and stress-related changes in behavioral responses.

Keywords Anxiety • Depression • Microglia activation • Monocyte trafficking • Psychosocial stress • Social defeat

Contents

1 Introduction

Psychosocial stress has been implicated in physiological and immunological alterations that contribute to the development of mental health disturbances such as anxiety and depression [1]. A plausible biological mechanism that contributes to stress-related mental health disorders is bidirectional communication between the immune system and the central nervous system (CNS) [2, 3]. Recent evidence suggests that stress-induced altered immune signaling significantly regulates mood and behavior [2, 4]. For example, chronic stress promotes a "transcriptional fingerprint" on peripheral leukocytes by up-regulating pro-inflammatory transcriptional control pathways, such as nuclear factor kappa B (NF-kB) [2, 5, 6]. Moreover, microglia activation, the innate immune cells of the brain, and sympathetic outflow to the peripheral immune system, as a consequence of stress, may facilitate the recruitment of inflammatory monocytes from the periphery to the CNS, thereby reinforcing stress-related behaviors [1]. Evidence from preclinical models supports a conserved inflammatory transcriptional response in leukocytes similar to that of chronically stressed humans [6]. Moreover, the establishment of anxiety-like behavior is promoted by trafficking of monocytes to the CNS [7]. It has also been postulated that anxiety-like behavior induced by long-term exposure of stress is reinforced through the propagation of cytokine signaling from the periphery to the brain [3]. The importance of elucidating the exact biological pathways that promote the development and exacerbation of anxiety and depression associated with

exposure to repeated psychosocial stress could be a potential avenue for the treatment of these disorders.

2 Repeated Social Defeat as a Model of Psychosocial Stress

The strong impact of social stress on humans' health led to the development of representative preclinical animal models [8–12]. There are several stress models that recapitulate elements of the pathophysiology of mental health disorders [3]. Some clinically relevant murine models include repeated social defeat (RSD) [3, 8], inescapable tail-shock [9, 12], restraint stress [10, 11], and chronic unpredictable stress or chronic mild stress [13–16]. RSD, a model of psychosocial stress in mice, provides a valuable and predictable probe to study the mechanisms leading to stress-related alterations in inflammation in both the periphery and CNS [17]. These inflammatory responses are frequently associated with anxiety- and depressive-like behaviors [7, 18, 19].

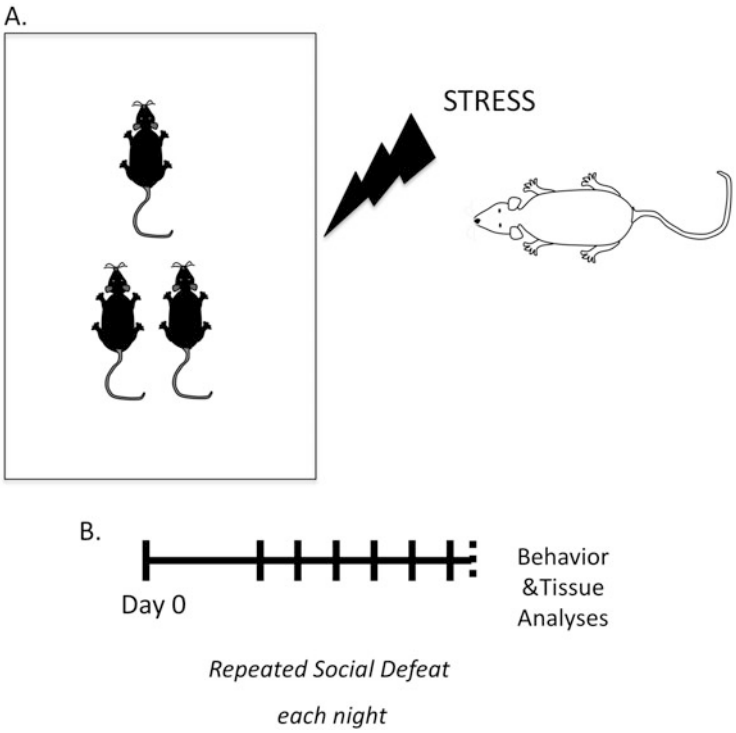

Fig. 1 Repeated social defeat (RSD): a model of psychosocial stress in mice. (**a**) During RSD, the social hierarchy is disrupted by introducing an aggressive male intruder into the cage of an established cohort of resident mice nightly for 2 h. (b) RSD in done once a day for six consecutive nights (cycles) to mimic a recurring stressor

The natural tendency of male mice living together in a cage is to form social hierarchies [8, 20]. During RSD, the social hierarchy is disrupted by introducing an aggressive male intruder into the cage of an established cage of resident mice for 2 h between 17:00 and 19:00 (Fig. 1a, b). After six consecutive nights, the resident mice are defeated and display submissive behaviors to the intruder [8, 20]. RSD elicits unique central, endocrine, and immune responses and invokes a series of individual differences in both inbred and outbred mice [18], suggesting that the paradigm is independent of a specific genetic background [21]. The RSD murine model of chronic stress provides a mean to study the associations between immune activation and behavioral changes indicative of anxiety-like and depressive-like phenotypes.

3 Psychological Stress Activates Neuroendocrine Pathways That Alter Immune Responses

Brain and immune system communication involves a bidirectional interaction between these two systems by a shared and specialized pathway known as the neuroimmune axis [1]. The neuroimmune cross talk cannot be discussed without the inclusion of the endocrine system as a major bridge that unifies the interactions between these two systems during homeostasis. The brain, endocrine, and immune system communicate with one another through the synthesis and release of endogenous chemical messengers, primarily cytokines, chemokines, neurotransmitters, and hormones [1]. Synchronized neuroendocrine and neuroimmune responses to infectious or stressful stimuli are mandatory for adaptive physiological and behavioral responses. For example, endogenous and exogenous stressors stimulate neuroendocrine, sympathetic, and immune responses, ensuring the activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS) [22]. HPA activation leads to the release of glucocorticoids (GCs) and SNS activation leads to the release of catecholamines, specifically epinephrine and norepinephrine, in circulation and tissues [19]. Jointly, HPA and SNS activation increases glucose breakdown, increases heart rate, and increases muscle tone during acute stress [23]. This provides increased energy availability to respond to aversive stimuli [22]. Through stimulation of the HPA and SNS, the stress response is relayed from the brain to peripheral organs and to the immune system with the purpose of activing the fight/flight response so that the organism can properly respond to the situation [1].

When homeostasis is disrupted by repeated psychosocial stress, there is an increase in inflammation in both the peripheral immune system and CNS by repeated activation of neuroendocrine and autonomic pathways [1]. The prolonged inflammatory state associated with social stress has the potential to contribute to the etiology of anxiety and depression [1]. Analysis of peripheral inflammatory

markers in the blood of patients with mood disorders reveals elevated levels of interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) levels [24–28]. In rodents, RSD causes an increase in pro-inflammatory molecules released into circulation [17]. Specifically, RSD causes an increase of IL-6, TNF-α, keratinocyte chemoattractant, macrophage inflammatory protein-2 (CCL8/MCP-2), and monocyte chemoattractant protein-1 (CCL2/MCP-1) [3]. It has been proposed that the increase of these pro-inflammatory factors is mediated by beta (β)-adrenergic signaling since pretreatment with propranolol, a non-selective β-adrenergic blocker, prevents increases of IL-6, TNFα, and CCL2 in circulation [22].

In terms of the mechanisms by which stress activates the inflammatory response, attention has focused on the impact of cytokines on the HPA axis, specifically related to major depression [2, 29, 30]. Administered cytokines stimulate the expression and release of corticotrophin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol, all of which are altered in depressed patients [2]. Likely, mice exposed to RSD have increased levels of pro-inflammatory cytokines in plasma, paralleled with an increased mRNA expression of CRH in the hypothalamus (HYPO), high blood levels of ACTH, and corticosterone in plasma [17, 31].

Repeated social defeat induces glucocorticoid insensitivity in innate immune cells [32]. This insensitivity prevents the GC-induced suppression of inflammation [3]. In the RSD model of stress, splenocytes did not have the ability to suppress NF-kB activity due to a failure of the nuclear translocation of the GC receptor [33]. Increased GC resistance may be related to high levels of cortisol in stress-related depression [34]. A recent study found that resistance to antidepressant treatment was associated with abnormalities in the HPA axis negative feedback response and the restoration of HPA axis hyperactivation to baseline levels was associated with remission in depressed patients [35]. A prolonged inflammatory state, as the one that occurs as a consequence of social stress, has the potential to contribute to the etiology of anxiety and depression [36, 37].

Evidence from in vivo studies show a clear trend to increase IL-6 plasma levels in both acute and chronic depression [29]. Monocytes of chronically stressed caregivers stimulated with LPS showed greater production of IL-6 relative to controls [38]. Interestingly, immune cells from patients that have undergone psychological stress do not show enhanced cytokine production in vitro, even though they show increased circulating levels of IL-6 [39]. The source of circulating IL-6 may derive from the CNS. Central injections to block IL-1β or adrenergic signaling attenuated stress-induced increase of circulating IL-6 [39]. There is evidence that psychosocial stress triggers activation of IL-6-containing neurons in the hypothalamo-neurohyphophyseal system, and this activation is paralleled by an increase in plasma IL-6 [39]. Studies indicate that magnocellular vasopressin neurons produce IL-6, and then it is transported to the neurohypophysis, where it is released into the peripheral circulation [40, 41]. Pretreatment with propranolol in mice subjected to RSD, and the use of IL-1 receptor type-1 knockout (IL-1R KO) mice in the RSD model, inhibited stress-associated increase in serum IL-6 comparable to home caged controls [42]. In the same manner, treatment with

antidepressant imipramine and benzodiazepines, lorazepam and clonazepam, significantly attenuated corticosterone and norepinephrine levels in plasma of mice subjected to RSD and decreased levels of IL-6 in plasma after six cycles of stress compared to home cage controls ([17]; unpublished data from Sheridan et al.). These data generated in different studies suggest that the production of IL-6 might also be from another cellular source different from peripheral immune cells.

4 Stress-Induced Neurobiological Dysfunctions Are Associated with Altered Behavioral Responses

Neurobiological and behavioral responses occur when physiological stress is interpreted within the brain leading to activation of fear and threat appraisal circuitry [7, 42]. Among the brain regions that are involved in the neurocircuitry of stress responses are the prefrontal cortex (PFC), HYPO, amygdala (AMYG), the CA3 and dentate gyrus of the hippocampus (HPC), and lateral septum (LS) [7, 17, 19]. Repeated activation of neurophysiological circuits by stress may lead to dysfunction in these limbic regions. In fact, dysregulation of the LS is associated with anxiety and depression in humans [43]. Other brain regions that are activated in response to social stressors are the bed nucleus of the stria terminalis (BNST) and nucleus accumbens (NAc). These limbic regions are implicated in regulating mood [42]. Studies suggest that anxiety- and depressive-like behaviors related to stress are associated with neurobiological dysfunctions within brain regions that regulate emotional and behavioral responses such as the PFC, AMYG, and HPC [42]. For example, using rodent stress models that induce depression- and anxiety-like symptoms, it was demonstrated that stress caused neuronal atrophy and dendritic retraction of neurons in the PFC [44]. Additionally, stress was related to dendritic atrophy and changes in spine density [28]. Social defeat stress was reported to reduce neurogenesis in the HPC [45]. Recent literature has identified structural and functional impairments within the brain's reward circuitry, specifically in the ventral tegmental (VTA) area to the NAc pathway, that were associated with anhedonia (decreased sucrose preference), a depressive-like symptom in rodents [28].

Recently, increasing attention has focused on the relationship between neuroinflammation and mood disorders [7, 17, 19, 42, 46]. As stated previously, bouts of repeated stress induce immune-enhancement and cause a pro-inflammatory phenotype by promoting a state of GC-insensitivity to develop in innate immune cells [8, 47]. This GC-insensitivity prevents the suppression of inflammation by reducing immune cell apoptosis and inhibiting NF-kB [3]. NF-kB is a downstream target of pro-inflammatory mediators IL-6, IL-1β, and TNF-α, both in peripheral tissues and in the brain. It has been shown that NF-kB has the potential of regulating the brain reward circuitry in depression models. Altered central levels of these pro-inflammatory factors act in the HPC, increasing depressive-like behavior in

response to chronic stress [28]. For instance, NF-kB activation in the HPC is required for the stress-related impairment of neurogenesis and induction of anhedonia [48]. Chronic social defeat stress increases levels of inhibitor of kappa B kinase (IKK) in the NAc, which increases NF-kB signaling by phosphorylation of IkB, causing its dissociation from NF-kB [28]. New immature excitatory spine structures on NAc dendrites are formed by the activation of NF-kB as well [49]. Moreover, pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α modulate neuronal activity [50]. For example, stress-induced neuroinflammatory signaling increases neuroplasticity that modifies connectivity between neuronal circuitry underlying behavioral disorders such as long-lasting anxiety and depressive symptoms [15, 48, 49].

In the RSD model, stress-induced neuroinflammation impaired hippocampal neurogenesis and promoted cognitive and affective behavioral deficits [51]. In this study, mice that were exposed to stress showed transient impairments in spatial memory recall that resolved within 28 days after stress cessation. In assessment of neurogenesis, the number of proliferating neural progenitor cells and the number of young, developing neurons were significantly impaired when examined 10 and 28 days after stress termination. In addition, social avoidance, a measure of depressive-like behavior associated with caudal hippocampal circuitry, persisted 28 days after RSD [51]. Treatment with minocycline, an antibiotic that has anti-inflammatory properties at the periphery and in the brain, prevented microglia activation and abrogated impairment in spatial memory after RSD. Minocycline did not prevent deficits in neurogenesis nor did it prevent the persistence of social avoidance behavior in mice exposed to stress [51]. These findings suggest that neuroinflammatory activation after psychosocial stress impairs spatial memory performance independent of deficits in neurogenesis and social avoidance [51].

5 Stress-Induced Microglia Activation Enhances Neuroinflammatory Signaling, Thereby Reinforcing Stress-Associated Behaviors

Stress activates microglia, the resident immune cells of the brain. The activation of microglia refers to changes in morphology (increased size of the soma, for instance) that corresponds with increased mRNA expression of pro-inflammatory cytokines and chemokines [1] (Fig. 2). Microglia plays a pivotal role in immune monitoring in the CNS, and when activated displays similar immune functions as peripheral macrophages, including the production of pro-inflammatory cytokines, chemokines, and prostaglandins [42]. Several studies have demonstrated that microglia are activated and increase neuroinflammatory signaling after stress exposure [7, 9, 19, 52–56]. Minocycline ameliorated the expression of IL-1 β in the brain after foot shock [57]. It was also reported that minocycline reduces restraint stress-induced cognitive deficits, depressive- and anxiety-like behaviors, the expression

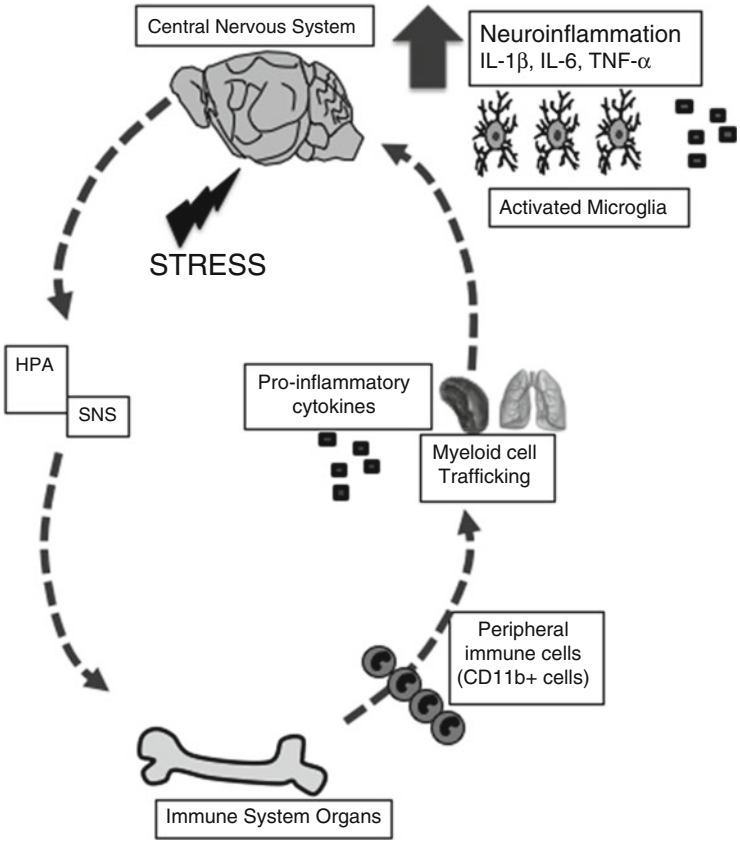

Fig. 2 Summary of stress-induced neuroimmune responses. RSD activates the HPA axis and the SNS, highlighted by increases in systemic glucocorticoids (GCs) that trigger the release of catecholamines. RSD enhances myelopoiesis and promotes the development, priming, and egress of GC-resistant CD11b+ cells from the BM to spleen, lung, and circulation. RSD promotes increased levels of pro-inflammatory molecules in plasma and in the CNS that promotes the development of a reactive endothelium. Vascular brain endothelial cells increase cell adhesion molecule expression that facilitates the adherence and extravasation of peripherally derived monocytes that differentiate into perivascular and parenchymal macrophages. The increased pro-inflammatory signaling is associated with behavioral alterations

of pro-inflammatory cytokines in the brain, and microglial activation [53]. Also, minocycline treatment attenuated neuronal activation induced by restraint stress as indicated by reduced Fos B labeling of neurons [53]. Thus, microglia likely contribute to the dysfunctional neurobiological stress response in the CNS by reinforcing neuronal activity. Moreover, there is evidence that activation of microglia potentiates HPA axis stimulation through the release of IL-1β within the HYPO [13], augmenting neuroendocrine outflow that may reinforce stress-associated behaviors.

It has been reported that long-lasting stress causes neuronal and microglia activation in stress-responsive brain regions such as the PFC, HYPO, AMYG, and the CA3 and dentate gyrus of the HPC [19, 56, 58, 59]. RSD evokes a "fight or flight" response that causes neuronal and microglia activation within brain regions associated with fear, anxiety, and threat appraisal [42]. It has been suggested that this neuronal activation precedes microglia activation. Evidence from the RSD stress model revealed an increase in region-specific expression of c-Fos indicating neuronal activation after just one cycle of social defeat, while Iba-1 expression, indicating microglial activation, occurred after three cycles of social defeat [19]. The increased mRNA expression of pro-inflammatory cytokines in microglia occurs generally after three cycles of RSD [7], suggesting that neuronal activation occurs first and is followed by activation of microglia. In support of this idea is the fact that microglia monitor neurons through CD200/CD200R interactions, chemokine signaling (i.e., CX3CL1), growth factors (i.e., M-CSF), ATP release, and neurotransmitter levels [1, 60–63]. In the RSD paradigm, region-specific microglia activation is associated with a reduction of neuronal-derived fractalkine ligand (CX3CL1) and decreased expression of fractalkine receptor (CX3CR1) on microglia, both anti-inflammatory regulators [1].

Altered morphological changes in microglia after RSD, foot shock, and chronic unpredictable stress were all associated with augmented mRNA expression of pro-inflammatory molecules and exaggerated pro-inflammatory responses to mitogen-stimulation ([9, 19, 46]). For instance, brain microglia from socially defeated mice show high levels of IL1-β, IL-6, TNF-α mRNA expression and reduced levels of GC responsive genes (GC-induced leucine zipper (GILZ) and FK506 binding protein-51 (FKBP51)) [19]. Furthermore, isolated brain microglia from RSD mice and cultured ex vivo produced increased levels of IL-6, TNF-α, and CCL-2 following stimulation with lipopolysaccharide (LPS) compared to microglia from the brain of home cage control mice [19], even 24 days after stress cessation [46]. Neuroinflammatory mediators such as IL1-β, TNF-α, and IL-6, are involved in the neurobiological changes that reinforce fear/anxiety and threat circuitry [42], promoting the development and maintenance of prolonged depressive- and anxiety-like behavior [42, 46].

Repeated social defeat-induced neuronal and microglia activation and active production of pro-inflammatory molecules promote the development of a reactive endothelium [42]. Vascular brain endothelial cells show increased cell adhesion molecule expression. This facilitates the adherence and extravasation of peripherally derived monocytes, which differentiate into perivascular and parenchymal macrophages [42]. Furthermore, parenchymal infiltration of peripherally derived monocytes is region specific and is observed within the fear, anxiety, and threat appraisal circuitry [7]. The increased accumulation of macrophages in the CNS is elicited by RSD enhanced neuroinflammatory signaling [7, 17, 19, 42]. RSD also enhanced reactivity of microglia and macrophages in a brain area-dependent manner [7, 19, 42]. In a previous study, reactivity of microglia and macrophages was determined through Iba-1 staining in the medial AMYG, PFC, and paraventricular nucleus (PVN) of the HPC. These findings show that social defeat

enhances the active microglia phenotype in several areas of the brain associated with fear and threat appraisal, after 6 days of RSD [19].

Repeated social defeat-induced anxiety-like behavior persists for at least 8 days after social defeat but resolves by 24 days [42]. Additionally, 24 days after RSD termination, markers of immune alterations associated with RSD, such as splenomegaly, plasma IL-6, and the number of circulating CD11b+ cells, return to control levels. Neuroinflammatory signaling returns to baseline by 24 days; however, the IL-6 mRNA level is still elevated at this time-point [7, 46]. Iba-1 labeling of microglia and increased Iba-1 proportional area were detected in the PFC 24 days after RSD. However, increased Iba-1 was no longer detected in the AMYG, CA3, and dentate gyrus of the HPC by 24 days, suggesting that the temporal dynamics of microglia activation is brain region-dependent. In addition, brain macrophage accumulation was no longer detected at 24 days after stress cessation [7]. It was apparent that microglia returned to a surveying state after RSD in a time- and region-dependent manner.

It should be noted, nevertheless, that social avoidance behavior developed after one cycle of social defeat [7], and long-lasting social avoidance to an intruder, was present 24 days after stress cessation when the majority of macrophages were no longer present in the CNS. Interestingly, administration of imipramine reversed social avoidant behavior in mice exposed to social defeat [46]. Moreover, 24 days of imipramine treatment in RSD mice significantly decreased stress-induced mRNA levels of IL-6 in brain microglia [46]. Following ex vivo LPS stimulation, microglia from mice exposed to RSD produced exaggerated levels of IL-6, TNF-α, and IL-1β, and this was reversed by imipramine treatment [46]. These data suggest that the antidepressant imipramine may exert its effect, in part, by down-regulating microglial activation and that social avoidance is mediated by long-lasting stress-induced phenotypic changes to microglia.

6 Stress-Induced Neuroendocrine Outflow Increases Release and Trafficking of Bone Marrow Derived Inflammatory Myeloid Progenitor Cells

Hypothalamic–pituitary–adrenal axis and SNS activation relay stress interpretation from the CNS to the peripheral immune system [1]. For example, stress leads to release of catecholamines into lymphoid tissues, including the BM, lymph nodes, and spleen [1, 64]. Peripheral immune cells express receptors for norepinephrine, and when these receptors are stimulated, functional responses occur that influence the development and mobility of these cells, as well as their inflammatory phenotype [6]. For example, repeated activation of the SNS caused an increase of norepinephrine in the BM that promoted a shift in myelopoiesis after RSD exposure [22]. Myeloid cells produced in the BM, as a result of RSD, were more inflammatory and less mature [31]. This is relevant since these inflammatory myeloid

progenitor cells (MPCs) traffic throughout the body and have an increased capacity of releasing pro-inflammatory cytokines upon becoming effector cells [1, 46]. The immature nature of these MPCs is pertinent since these cells were found to be GC-resistant [31]. It has been suggested that the accumulation of these GC-insensitive, immature monocytes corresponds with a state of GC-insensitivity during chronic stress [1]. Pharmacological blockade of stress-induced myelopoiesis by β-adrenergic antagonism prevented accumulation of monocytes [6, 22, 65], and this was associated with reversal of GC-insensitivity [22] and abrogation of pro-inflammatory transcriptional profiles following RSD exposure [6].

BM-derived monocytes traffic and are recruited into inflamed tissue in models of trauma, neurological disease and infection [66, 67]. Evidence also indicates that trafficking and recruitment of monocytes from the periphery into the brain occur after exposure to psychological stress [7, 19, 42, 68, 69], representing a mechanism by which the immune system communicates with the brain. Following 5 days of foot shock stress, BM-derived monocytes trafficked to the ventral HPC and demonstrated a ramified microglia-like morphological phenotype [70]. The stimulus of simply watching other mice receiving foot shock stress was enough to recruit monocytes to the brain parenchyma in another study using rodents [68]. Chemokine receptor 2 (CCR2) and β-adrenergic signaling contributed significantly to the infiltration of BM-derived monocytes [68, 69]. In a preclinical model of neuropathic pain, partial sciatic nerve ligation induced anxiety-like behavior and trafficking of BM-derived monocytes into the AMYG [69]. In this same study, administration of CCR2 and IL-1 receptor antagonist in the AMYG ablated monocyte trafficking [69]. Stress-induced trafficking of inflammatory monocytes exacerbates the inflammatory signaling in the CNS and influences behavior [7, 22, 42, 65, 69]. For instance, in a clinical study of depression, patients who committed suicide had increased Iba-1 immunolabeling compared to controls, indicating the presence of vascular-associated macrophages in the brain [71].

Psychological stress causes distinct patterns of monocyte trafficking to the CNS [3]. Green fluorescent protein-positive (GFP+) BM-chimeric mice were created in order to evaluate the brain regions that these cells infiltrated following stress exposure, by reconstituting the BM of wild-type mice with BM-derived donor cells that ubiquitously expressed GFP [7]. Consistent with previous studies demonstrating that microglia are not BM derived under homeostatic conditions [63] control chimeric mice displayed little or no GFP+ cells within the CNS parenchyma [7]. Nonetheless, RSD mice displayed GFP+ BM-derived monocytes that extravasated into the brain parenchyma in a region- and defeat cycle-dependent manner [7]. These GFP+ parenchymal macrophages showed a ramified, microglia-like morphology with elevated expression of Iba1 [7].

The increased infiltration of parenchymal ramified GFP+ macrophage was observed following six cycles of RSD [7]. Increased ramified GFP+ cells were observed in brain regions associated with fear, anxiety, and threat appraisal including the PFC, PVN, LS, BNST, and AMYG, but were not observed in other brain regions like the motor cortex, striatum, somatosensory cortex, or cerebellum [7]. This regional specificity of RSD-induced trafficking reinforced the patterns

of c-Fos and Iba1 expression, neuronal and microglia activation respectively, observed in other studies [7, 19]. These data suggest that stress tends to cause region-specific monocyte recruitment to the brain.

7 Stress-Induced Brain-Monocyte Trafficking Influences Behavior

Clinical and preclinical studies have indicated that stress promotes the onset of anxiety- and depressive-like behaviors [72–74]. There are temporal relationships between the development, resolution, and recurrence of anxiety and neuroimmune signaling in the RSD model. For example, anxiety and pro-inflammatory cytokine production in the CNS, and brain macrophage accumulation were observed in an exposure dependent manner after stress exposure. These parameters were increased moderately compared to controls after three cycles or RSD and peaked after six cycles of social defeat [7]. The resolution of anxiety and brain-monocyte trafficking was also correlated [1]. For example, as stated earlier in this chapter, both anxiety-like behavior and increased brain macrophages persisted for at least 8 days after RSD and both of these parameters were resolved by 24 days [42]. It should be noted that social avoidance to an aggressor mouse was maintained 24 days later [42]. Based on this timing and lack of macrophages in the CNS 24 days after stress cessation, social avoidance is present independent of monocyte trafficking to the CNS.

Experimental interventions in the RSD model helped elucidate the cause and effect relationship between anxiety-like behavior and brain-monocyte trafficking to the brain [19, 75]. For instance, pretreatment with propranolol prevented the release and trafficking of BM-derived monocytes to the CNS [19], and this was associated with reversal of anxiety-like behavior measured in the open field and light/dark box tests. Moreover, IL-1R1KO mice prevented both RSD-induced anxiety-like behavior and brain-monocyte trafficking [19, 42]. Together, these data support the notion that RSD-induced anxiety-like behavior is dependent upon monocyte trafficking to the brain and is mediated by SNS and IL-1 signaling.

In subsequent studies, interference with monocyte trafficking to the CNS ablated anxiety-like behavior in mice exposed to stress [7]. Transgenic mice deficient in CCR2 (CCL2 receptor KO) or CX3CR1 (CX3CR1KO), which are two key monocyte chemokine receptors, were used to show that the expression of both receptors is required for RSD-induced trafficking of monocytes to the CNS [7]. In this study, monocyte trafficking into the brain and anxiety-like behavior following RSD were absent in both CCR2KO and CX3CR1KO mice [7]. A key finding is that chemokine deficiency was associated with blockade of stress-induced accumulation of both $CD45^{hi}$ perivascular macrophages and GFP+ parenchymal macrophages, as studied in both naïve and BM-chimeric mice [1, 7]. However, increased circulating LY6Chi monocytes were detected in CCR2 and CX3CR1KO mice after RSD, suggesting

that the release of monocytes is not sufficient to traffic into the brain. Prevention of brain-monocyte trafficking in knockout mice was not associated with attenuation of elevated IL-1β mRNA expression in mice exposed to stress [7], suggesting that intrinsic neuroinflammatory signaling is not enough to promote anxiety-like symptoms. Thus, this finding indicates once more that monocyte trafficking to the brain is likely responsible for the promotion of anxiety-like behavior following RSD.

Other studies have also supported the hypothesis that brain macrophage trafficking affects behavior [69, 76]. For example, in an inflammatory liver disease model, an important role for brain macrophage trafficking in the development of fatigue and depressive-like behavior was demonstrated [76]. Also, in a neuropathic pain model, region-specific trafficking of monocytes to the AMYG was CCR2/CCL2-dependent and monocyte trafficking was found to exacerbate pain-induced anxiety-like behavior [69]. Moreover, in this same study, pain-induced anxiety-like behavior associated with monocyte trafficking to the AMYG was prevented by local microinjection of IL-1R1 antagonist [69]. Likewise, in a model of inflammatory liver disease, recruitment of TNF-alpha-expressing monocytes to the brain promoted depressive-like behavior and fatigue [77]. These reports suggest that monocyte trafficking to the brain promotes changes in behavior through central cytokine signaling as well.

8 Concluding Remarks

As it has been consistently shown, chronic psychosocial stress promotes activation of peripheral and central myeloid cells (macrophages/microglia) that enhance neuroinflammatory signaling and contribute to the development of anxiety-like behavior, and maintenance of long-lasting social withdrawal [3, 7, 17, 19, 22, 42, 46]. Investigators have suggested that dysfunctional neuron–microglia crosstalk may be the root of neuroinflammatory signaling after stress exposure [78]. Previous work demonstrated that RSD triggered anxiety-like behaviors and enhanced the inflammatory state in the periphery and in the CNS in a β-adrenergic dependent manner [19, 22]. Interruption of noradrenergic locus coeruleus projections was accompanied by a reduction in stress-induced IL-1β production [54]. In other words, central noradrenergic responses exert a major role in stress-induced microglia activation.

Preclinical animal models, such as the RSD paradigm, have provided insights into mechanisms by which stress impacts neuroimmune function, its dysregulation, and also behavior. In summary (Fig. 2), the proposed mechanism is that RSD evokes a "fight or flight" response that promotes neuronal and microglia activation to co-occur. RSD activates the HPA axis and SNS activation highlighted by increased levels of corticosterone in serum, pro-inflammatory products in plasma (IL-6 and CCL-2), and catecholamines in tissues and circulation. The increased levels of norepinephrine in the bone marrow specifically have been associated with a shift in myelopoiesis promoting the development, priming, and egress of an MPC

from the bone marrow to circulation and organs (spleen, lung, and brain). RSD promotes a reactive endothelium stimulating the extravasation of peripherally derived monocytes [79]. The trafficking of monocytes to the perivascular spaces and parenchyma is region specific and co-occurs within brain regions associated with fear, anxiety, and threat appraisal circuitry [1, 7, 19]. These monocytes differentiate into macrophages enhancing neuroinflammatory signaling which is associated with prolonged anxiety- and depressive-like behaviors [1, 7, 17, 19, 46]. Overall, these translational insights may be useful in generating new therapeutic approaches for those patients suffering from anxiety and depression.

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Inflammation Effects on Brain Glutamate in Depression: Mechanistic Considerations and Treatment Implications

Ebrahim Haroon and Andrew H. Miller

Abstract There has been increasing interest in the role of glutamate in mood disorders, especially given the profound effect of the glutamate receptor antagonist ketamine in improving depressive symptoms in patients with treatment-resistant depression. One pathway by which glutamate alterations may occur in mood disorders involves inflammation. Increased inflammation has been observed in a significant subgroup of patients with mood disorders, and inflammatory cytokines have been shown to influence glutamate metabolism through effects on astrocytes and microglia. In addition, the administration of the inflammatory cytokine interferon-alpha has been shown to increase brain glutamate in the basal ganglia and dorsal anterior cingulate cortex as measured by magnetic resonance spectroscopy (MRS). Moreover, MRS studies in patients with major depressive disorder have revealed that increased markers of inflammation including C-reactive protein correlate with increased basal ganglia glutamate, which in turn was associated with anhedonia and psychomotor retardation. Finally, human and laboratory animal studies have shown that the response to glutamate antagonists such as ketamine is predicted by increased inflammatory cytokines. Taken together, these data make a strong case that inflammation may influence glutamate metabolism to alter behavior, leading to depressive symptoms including anhedonia and psychomotor slowing.

Keywords Cytokines • Depression • Glia • Glutamate • Inflammation • Magnetic resonance imaging • Neuroimaging

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Contents

1 Introduction

Major depressive disorder (MDD) is a common and devastating disease, being the leading cause of disability worldwide and the tenth leading cause of death (by suicide) in the United States [1]. During 2009–2012, 7.6% of Americans aged 12 and over (~20 million) exhibited persistent moderate or severe depressive symptoms warranting some form of antidepressant therapy [2]. Unfortunately, approximately 1/3 of depressed patients fail to respond to conventional, monoamine-targeted therapies [3], and therefore, there is a pressing need to elaborate new conceptual frameworks and new targets for the development of novel therapies to treat depression, especially those who are treatment resistant. Relevant in this regard, there has been increasing interest in the role of inflammation and glutamate metabolism as two major new pathways to pathology that may ultimately address the shortcomings of the current monoamine hypothesis of depression [4–11]. Although the development of treatments targeting inflammation and glutamate has largely proceeded along independent paths, increasing data suggests that there may be a convergence of these two mechanisms of depression [5, 9, 12]. This intriguing possibility opens the door to a variety of therapeutic considerations, both in terms of the development of novel immune biomarkers that can predict response to glutamate-targeted therapies but also in terms of the development of treatments that address inflammation and glutamate metabolism, which can be combined or proceed in tandem. This chapter will address the data that supports the notion that inflammation and glutamate metabolism may be intimately intertwined in their impact on behavior, particularly as it relates to MDD. We will describe the mechanisms that link these pathways as well as explore the potential translational implications.

2 Role of Inflammation in Depression

There are multiple lines of evidence that support the notion that inflammation can contribute to the symptoms that characterize MDD [4, 7, 9]. Patients with MDD have been found to exhibit increased markers of inflammation as well as activation of inflammatory signaling pathways in the peripheral blood, cerebrospinal fluid (CSF), and postmortem brain samples [4, 7, 9, 13]. Meta-analyses of this literature have revealed that the peripheral blood cytokines interleukin (IL)-6 and tumor necrosis factor (TNF) and the acute phase reactant C-reactive protein (CRP) are the most reliably elevated in MDD patients [14–16]. Moreover, polymorphisms in genes associated with the inflammatory response have been shown to predict the development of depression as well as the response to conventional antidepressant medication [17, 18]. Interestingly, conditions associated with increased inflammation including obesity, childhood trauma, and medical illness have also been linked with reduced response to antidepressants [19–23]. In addition, administration of inflammatory stimuli including inflammatory cytokines (i.e., interferon (IFN)-alpha), endotoxin, and typhoid vaccination has been shown to induce depressive symptoms in humans [24–28]. Patients with medical disorders that exhibit increased inflammation including autoimmune and inflammatory disorders, cardiovascular disease, metabolic disorders, and cancer also report increased depressive symptoms compared to otherwise healthy individuals [29]. Finally, a developing literature indicates that blockade of inflammation can reduce depressive symptoms [30]. Of note, this data largely derives from patients with autoimmune or inflammatory disorders receiving anti-inflammatory therapies including anti-cytokine antibodies, who do not carry a diagnosis of MDD [30]. The literature on the use of anti-inflammatory agents in MDD is much smaller and less convincing [30–36], although as this research area evolves, the parameters of intelligent trial design are becoming more clear (see below), and new studies will ultimately reveal the utility of anti-inflammatory treatment strategies in MDD patients [37].

3 Glutamate and Mood Disorders

In order to measure glutamate in the brain of patients with mood disorders, studies have used proton magnetic resonance spectroscopy (MRS). MRS technology is based on the fact that magnetic resonance acquisition involves the measurement of echoed resonance frequency waves of multiple cellular chemical constituents [38]. The individual chemical and metabolite constituents can be measured by suppressing the resonance frequency of water molecules. MRS uses this information to progressively filter out more abundant metabolites to study metabolites that exist at lower concentrations but above 1 mM [38–40]. MRS is the only in vivo method available to measure glutamate in humans [38–40]. Although MRS cannot distinguish extracellular from intracellular glutamate and is in many studies unable

to distinguish glutamate from its precursor glutamine; two recent studies have demonstrated that concentrations of glutamate + glutamine (Glx) estimated using MRS were significantly correlated with cortical excitability – measured using transcranial magnetic stimulation, thus validating the contention that MRS measures of glutamate are indeed associated with neural activity [41, 42].

MRS-based studies of glutamate and/or Glx in unipolar major depression have demonstrated widely variable findings likely attributable to the heterogeneity that plagues the diagnosis and classification of the disorder [43, 44]. Accordingly, some studies have reported increases in brain glutamate including increases in the basal ganglia [45], while the majority of studies have shown decreases in glutamate, typically measured as Glx [43, 44, 46, 47]. One meta-analysis using pooled MRS findings from multiple studies failed to discern meaningful and consistent glutamate changes in unipolar major depression [43], while another meta-analysis with a more restricted inclusion of studies that provided absolute Glx values reported an association between unipolar depression and decreased Glx in the prefrontal cortex [48]. Decreases in Glx have been reported to normalize following various treatments for depression including electroconvulsive therapy [49–51], the serotonin reuptake inhibitor citalopram [52], and total sleep deprivation [53]. Interestingly, treatment with subanesthetic doses of ketamine, a glutamate receptor (*N*-methyl-D-aspartate, NMDA) antagonist, was not associated with Glx changes in the anterior cingulate cortex despite dramatic improvements in mood [54]. It has been proposed that the timing of MRS scanning missed a critical but narrow time window when ketamine effects on glutamate are most pronounced [55]. Alternately, it is possible that the ketamine-induced alterations in glutamate concentrations were below the sensitivity of detection by MRS. Moreover, ketamine effects on other glutamate-active biomolecules such as quinolinic acid (QA) may be responsible for its antidepressant response [56]. Nevertheless, data saying that a glutamate antagonist can treat major depression so dramatically raises questions regarding the validity and reliability of Glx measures in depression. Indeed, it should be noted that proton MRS studies may miss potential glial contributions to glutamate dysregulation. 13C MRS may address some these issues, given that it tracks the components of glutamate flux and cycling in neuronal and glial cell compartments by the administration of 13C-labeled glucose, which is metabolized in both neurons and glia, combined with the administration of 13C-labeled acetate, which is metabolized exclusively in glia [57, 58].

In contrast to unipolar depression, the phenotypically and biologically better defined bipolar depression has been consistently associated with increased glutamate in anterior cingulate regions as measured by MRS. In fact, increased glutamate in the anterior cingulate cortex has been one of the most consistent neuroimaging findings in bipolar disorder and has been supported by at least one large meta-analysis [59].

Another strategy to address alterations in glutamate neurotransmission in depressed patients has been the use of postmortem studies. Decreased number and functioning of glutamate transporters on the surface of astrocytes have been reported in specimens from depressed subjects [60–63], and gene expression

studies have indicated evidence of significant astrocyte dysfunction and glutamate dysregulation in depressed suicides [63–65]. In addition, loss of astrocytes and oligodendrocytes, both of which play a central role in glutamate clearance (see below), is one of the most reliable findings regarding cell loss in the brain of depressed individuals [62, 63, 66, 67]. Postmortem studies have also reported downregulation of several genes associated with synthesis of glutamate transporters in the brain including SLC1A2, SLC1A3, and L-glutamate-ammonia ligase (GLUL) in patients with depression [60, 68]. Moreover, decreased expression of glutamine synthetase (the astrocytic enzyme that detoxifies glutamate by converting it into glutamine) has also been detected in postmortem studies of depressed suicide victims [60, 67, 69, 70]. Finally, a single-nucleotide polymorphism within the NMDA receptor gene (GRIN2B) rs1805502 has been shown to confer increased susceptibility to treatment-refractory depression (TRD) compared to non-TRD groups [71].

Laboratory animal models have also supported a role of glutamate dysregulation in depressive-like behavior, especially in the context of acute and chronic stress. For example, inhibition of astroglial surface glutamate transporters by chronic stress (or pharmacologic inhibition or genetic deletion) has been shown to lead to anhedonia – a core symptom of depression [62, 72, 73]. In addition, acute stress has been shown to increase trafficking, recycling, and expression of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors at the postsynaptic nerve terminal, leading to potentiation of glutamate neurotransmission [73]. Chronic stress may also impair the ability to effectively clear synaptic glutamate through glial excitatory amino acid transporters (EAATs) leading to glutamate accumulation, spillover, increased activation of extrasynaptic glutamate receptors, and ultimately excitotoxicity – a process that has been proposed to underlie several neuropsychiatric and neurodegenerative disorders [73]. Finally, chronic stress has been found to decrease the rates of flux through the glutamate-glutamine cycle, resulting in reduced glutamate breakdown and greater glutamate stagnation and buildup [73]. Thus, laboratory animal models of stress-induced depressive-like behavior are reliably associated with excessive glutamate neurotransmission and excitotoxicity.

4 Impact of Inflammation on Glutamate Neurotransmission

Given the notion that both inflammation and glutamate neurotransmission play a role in depression in humans and laboratory animal models, it is important to consider the basic science literature that supports that these two processes interact. Therefore, we will first briefly describe the cellular and molecular constituents of glutamate neurotransmission followed by a consideration of how inflammation may affect these processes.

4.1 Cellular Regulation of Glutamate Release and Reuptake

Glial cells including astrocytes, oligodendrocytes, and microglia play a key role in glutamate neurotransmission. Glial cells are known to sense and respond to glutamate secreted by neurons via surface glutamate receptors. Table 1 presents a detailed overview of the surface localization and functioning of glutamate receptors on glial cells. The extracellular concentration of glutamate depends upon a balance between glutamate release from neural, astrocytic, oligodendrocytic, and microglial sources and its clearance mechanisms [74–76], all of which can be significantly influenced by inflammatory mechanisms [5, 77] (see below).

Glutamate release occurs through several channels including vesicular discharge from neurons during neurotransmission or from astrocytes during gliotransmission (Fig. 1). Intra-synaptic glutamate removal involves transporter-mediated uptake by EAATs into the cytoplasm of the astrocytes and oligodendrocytes, where it is rapidly detoxified by conversion to glutamine by the enzyme glutamine synthetase [78–84]. EAATs are specialized transport proteins expressed on the perisynaptic surface of glial cells (astrocytes, oligodendrocytes, activated microglia, and neurons) that remove glutamate from the synaptic and to a lesser extent from the extrasynaptic space [85]. There at least five different types of EAATs, with EAAT1 mostly located in oligodendrocytes, EAAT2 in the synaptic regions of the astrocytes, and EAAT3, EAAT4, and EAAT5 in neurons.

Under pathological conditions, excessive glutamate can accumulate through a number of mechanisms including (1) release through anion channels; (2) reverse efflux through EAATs; (3) excessive release via the cystine-glutamate exchange transporter (system Xc- SLC7A11), which exchanges extracellular cystine for intracellular glutamate (1:1) to ultimately form the antioxidant glutathione (GSH); (4) inter-glial glutamate transfer through gap junctions (connexin channels); and (5) pathological release through purine-controlled ion channels (P2X7) [79] (see Fig. 1). While glutamate release from EAATs (reverse efflux) and presynaptic neurons is primarily located within the synapse, other forms of glutamate release such as from system Xc- transporters and anion and connexin channels on the surface of glial cells can occur within the extrasynaptic space [79]. Excess synaptic glutamate can lead to profound excitotoxicity and cell death by triggering apoptotic mechanisms [86], overexciting ionotropic glutamate receptors (NMDA, AMPA, kainate) [87], and paralyzing system Xc- transporters which neutralize oxidative stress via GSH synthesis [78]. Extrasynaptic glutamate is equally toxic as it engages the extrasynaptic NMDA receptors, leading to suppression of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) [88, 89]. Thus, the mechanisms to clear and remove glutamate are of great importance [74, 84]. Of note, removal of extrasynaptic glutamate can be mediated by transport of glutamate across the blood-brain barrier (BBB) by EAATs located on the cerebral blood vessels into the peripheral blood circulation [5, 90]. Finally, additional influences on glutamate neurotransmission and excitotoxicity can occur

Table 1 Glutamate receptor/transporter expression on glia

Receptor/ transporter	Microglia	Oligodendrocyte	Astrocyte
AMPA	• Only in activated (M1) state • Morphology change • Promotes chemotaxis • Releases TNF-alpha	• On surface of cell body • Senses signaling from axons • Oligodendrocyte progenitor differentiation	• Ubiquitous (all regions) • Subunits vary between regions • Mostly AMPA A1 and lacking AMPA A2 subunits
Kainate	• Identical to AMPA • All subunits expressed • Mediates inflammatory functions	• On surface of cell body	• No functional expression • Immunostaining has revealed some evidence
NMDA	• Functionally expressed • Subunits: NR1, NR2A, NR2B, NR3 • Immunostaining supported	• On cell process only • Less susceptible to Mg^{2+} block • Weaker Ca^{2+} permeability	• Expressed on cell surface • Weaker Mg^{2+} blockade • Lower Ca^{2+} permeability • Different from neuronal NMDA
Group 1 - mGluR Types 1 and 5	• mGluR Type 1 not reported • PLC/IP3 transduction • Has anti-inflammatory effects	• Insufficient data	• PLC/IP3 transduction • mGluR1 type 5 > mGluR Type 2 • Has anti-inflammatory effects
mGluR, Group 2 (R2,3)	• Adenyl cyclase transduction • Proinflammatory effect • Neurotoxic phenotype	• Insufficient data	• Adenyl cyclase transduction • Mostly mGluR3. • Regulation of EAAT
mGluR, Group 3 (R4, 6–8)	• mGluR4,5,8 (not 7), • Adenyl cyclase transduced • Neuroprotective • Decreases stress-induced activation of Group 2 mGluRs	• Not activated by synaptic glutamate	• Probably not expressed
GLAST/EAAT1	• Only during activation • Neurotrauma • Neurodegeneration	• Myelinating oligodendrocytes • Blockade results in severe toxicity to oligodendrocytes	• Probably not expressed
system Xc- transporter	• Responds to demand for GSH • Responds to oxidative stress • Releases glutamate	• Expressed similar to astrocytes	• Responds to demand for GSH • Responds to oxidative stress • Releases glutamate

(continued)

Table 1 (continued)

Receptor/ transporter	Microglia	Oligodendrocyte	Astrocyte
Glutamate-permeable volume-regulated anion channels	• Expressed upon immune stimulation		• Excretes/extrudes glutamate • Anionic swelling • Possible hypo-osmotic states (hepatic encephalopathy)

AMPA alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, *Ca* calcium, *GLAST* glutamate aspartate transporter, *GSH* glutathione, *IP3* inositol triphosphate, *Mg* magnesium, *mGluR* metabotropic glutamate receptor, *NMDA N*-methyl-D aspartate, *PLC* phospholipase C, *TNF* tumor necrosis factor, *system Xc transporter* cystine-glutamate exchange transporter

through metabolic mechanisms such as the activation of kynurenine (KYN) pathway (described below) [77, 91].

4.2 *Immunologic Influences on Glutamate Neurotransmission*

Inflammatory processes impact almost all aspects of glutamate neurotransmission including multiple cellular effects that influence both glutamate release and reuptake mechanisms. In addition, inflammation activates metabolic pathways involving indoleamine 2,3 dioxygenase (IDO) and the KYN pathway that generates neuroactive metabolites which can also affect glutamate metabolism. These effects of inflammation on glutamate neurotransmission will be reviewed below:

4.2.1 Glutamate Release

Vesicular release by astrocytes: Presynaptic neuronal release is the foundation of excitatory neurotransmission and can show dramatic increases during conditions of stress [92]. Physiologically, astrocytes sense synaptic release of glutamate through their surface receptors and transporters and respond by triggering internal Ca^{2+} fluxes of their own [93]. These fluxes are communicated to other astrocytic cells through physical connections known as gap junctions that are made of specialized proteins known as connexins and permit cell-to-cell transfer of ions and glutamate across the astroglial network [85, 94, 95]. This physiological process referred to as "gliotransmission" involves vesicular release and transfer of glutamate, ion fluxes, and excitatory signals in a transsynaptic manner to maintain tonic regional excitatory activity and is made possible by the anatomically interconnected astroglial network or syncytia [93]. Interestingly, cytokines such as TNF and IL-1-beta may amplify and alter the magnitude and propagation of intracellular Ca^{2+} oscillations

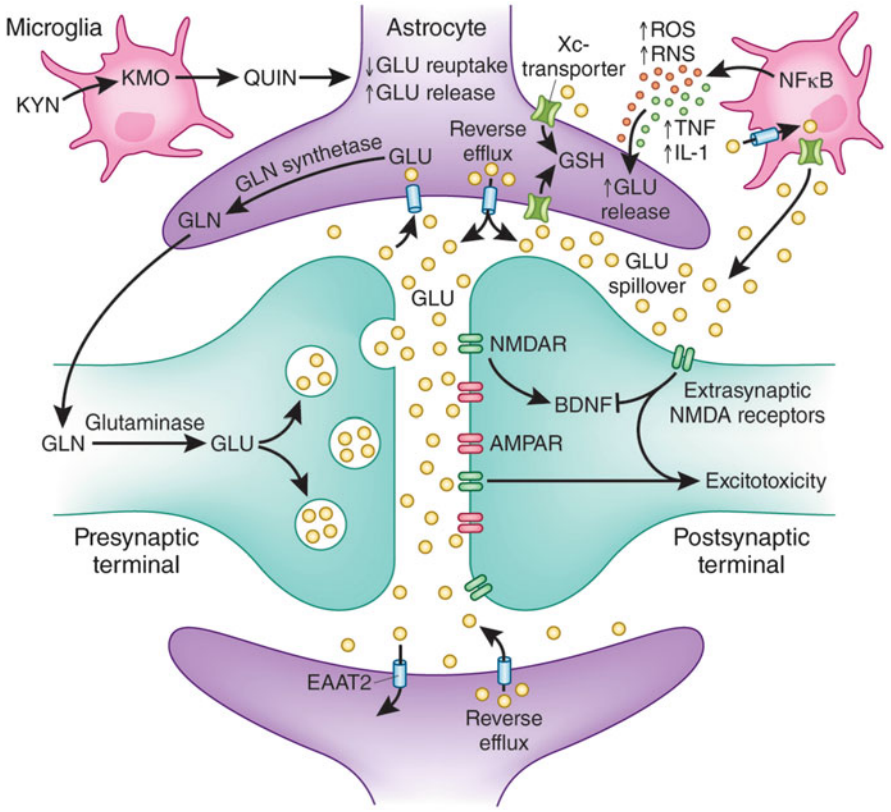

Fig. 1 The impact of inflammation on glutamate regulation in the tripartite synapse. Activation of the inflammatory response including microglia and macrophages in the brain leads to the release of inflammatory cytokines including interleukin-1-beta (IL-1) and tumor necrosis factor (TNF). These inflammatory proteins and their signaling pathways including nuclear factor kappa B (NF- κB) can reduce the expression and function of excitatory amino acid transporters 2 (EAAT2) on astrocytes and cause reverse efflux of glutamate (GLU) through EAAT2. In addition, microglia and macrophages produce reactive oxygen and nitrogen species (ROS and RNS, respectively) that increase oxidative stress, driving the production of glutathione (GSH), which is synthesized from cystine imported into astrocytes, oligodendrocytes, and microglia in exchange for glutamate (1:1) by the cystine-glutamate exchange system Xc- transporter, further increasing extracellular glutamate buildup. Synaptic glutamate taken into astrocytes is converted by GLN synthetase to glutamine (GLN) that is released and taken up by presynaptic neurons and converted back into GLU by glutaminase where it is packaged in synaptic vesicles and released back into the synapse. Conversion of kynurenine (KYN) into quinolinic acid (QUIN) in microglia and macrophages via the enzyme kynurenine 3-monooxygenase (KMO) further contributes to excessive glutamate signaling through receptors for *N*-methyl-D-aspartate (NMDAR), kainate, and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR) by decreasing astrocytic glutamate reuptake and stimulating glutamate release. Glutamate spillover into the extrasynaptic space can activate extrasynaptic NMDA receptors, leading to reduced neurotrophic factors including brain-derived neurotrophic factor (BDNF), and in combination with excessive synaptic NMDAR signaling ultimately contributes to excitotoxicity and synaptic destruction

to transfer immune signals and to mediate changes in neural activity [96]. Thus, the impact of inflammatory cytokines on gliotransmission may be an important mechanism for increased glutamate release under pathological conditions.

Anion channel release: The mechanism of glutamate release through anion channels is believed to occur under conditions of astrocytic toxicity characterized by astrocytic swelling and edema as what happens during stroke and hepatic encephalopathy and can result in profound neurotoxicity and neuronal loss [82].

Reverse efflux: Normally glutamate flows from the extracellular space into astrocytic cytoplasm to be detoxified, but under pathological contexts, a reverse operation of the EAATs results in a retrograde efflux of glutamate from the astrocyte into the cytoplasm [79, 84]. This reverse flow is thought to result from the inflammatory activation of cyclooxygenase (COX)-2 resulting in prostaglandin E2 synthesis which can increase calcium mobilization from their intracellular stores [80, 97].

Release through system Xc- transporters: The system Xc- transporter is a membrane transport system on glial cells that releases glutamate into the extracellular space in exchange for intracellular transport of cystine, which acts as a substrate for the synthesis of GSH after being metabolized to cysteine [78, 98]. This mechanism is highly vulnerable to immune influences and could lead to serious glutamate toxicity during inflammatory activation [5]. Inflammatory cytokines precipitate oxidative stress [5, 99] accelerating the rate of GSH synthesis which in turn leads to greater absorption of cystine coupled with greater releases of glutamate into the synapse [5]. In addition to promoting excitotoxicity, toxic levels of glutamate in the synapse can lead to a toxic paralysis of the system Xc- transporters (oxidative glutamate toxicity) leading to extra synthesis and expression of these transporters in the peri- and non-synaptic regions of the astrocytes. This in turn leads to extrasynaptic release of glutamate that binds to the extrasynaptic NMDA systems leading to growth factor suppression and toxicity [88, 89].

Microglial glutamate release: When activated, M1 microglia express glutamate receptors (see Table 1), which when bound by glutamate can lead to marked glutamate release [100, 101]. M1 microglia have also been shown to express EAATs on their surface when activated by inflammatory stimuli [e.g., lipopolysaccharide (LPS)] [102] or excessive stress-induced neural activity [92, 103, 104]. In addition, M1 microglia (and macrophages) also express glutamine synthetase and the system Xc- transporter [105]. Of note, EAATs, glutamine synthetase, and system Xc- transporters in microglia and macrophages are not constitutively expressed like in astrocytes but are induced during immune activation [105]. The precise biological purpose of EAAT expression by microglia is unclear, but under physiological conditions, it can reinforce glutamate clearance by astrocytes [105]. However, in pathological states as seen during immune activation, the need for GSH increases, and thus glutamate is released through the system Xc- transporter in exchange for cystine [105]. Moreover, glutamate released by microglia can be transferred through connexin channels in gap junctions into astrocytes, where it has been shown to suppress synthesis of EAATs [101],

contributing to impaired glutamate reuptake (see below) and possibly further adding to the overall toxic glutamate load [5].

Purinergic mechanisms: Adenosine triphosphate (ATP) is released by dead and damaged tissue as well as by psychological stress and can activate profound cytotoxic immune responses through the intracytoplasmic "inflammasome" systems [9, 106, 107]. ATP can also lead to abrupt release of glutamate through anion channels exclusively controlled by the purinergic P2X7 receptor [108]. Thus, the release of ATP during stress can both activate inflammatory pathways and the production of IL-1-beta and IL-18 via the inflammasome and also stimulate the release of glutamate through the P2X7 receptor, which thereby serves as an intriguing point of convergence between inflammation and glutamate.

4.2.2 Glutamate Reuptake/Clearance

EAATs: EAAT genes have promoter regions that are responsive to immune regulation through nuclear factor kappa B and other signaling pathways, ultimately leading to decreased functional activity and expression of EAATs [109]. For example, cultured astrocytes exposed to IL-1-beta and TNF as well as tissue samples from inflammatory CNS lesions of both laboratory animals and humans demonstrated decreased expression and function of GLT1/EAAT1 and EAAT2 transporters [82, 83, 110–113]. The EAAT system functions mostly within the confines of the tripartite synaptic space which is enveloped by astrocytic processes that forms the astrocytic "cradle." This astrocytic cradle serves to prevent "spillover" of glutamate into the extrasynaptic space [114].

Glutamate transport across BBB: Glutamate released into the extracellular and extrasynaptic space can be highly toxic and needs to be cleared expeditiously. Though glutamate removal by EAATs is sufficient to prevent excitotoxicity from glutamate released under physiological conditions [87, 115], their transport activity is often inadequate under pathological conditions such as during significant immune activation and glutamate buildup [5, 90]. As noted above, glutamate can be removed from the brain by channeling it into the peripheral blood by EAATs associated with the BBB [5, 90]. Of note, because this brain-to-blood "glutamate scavenging" mechanism is based on EAAT activity, it is vulnerable to the inhibitory effects of inflammatory mediators as described earlier [5, 90]. Conversely, the activity of this clearance mechanism can be potentiated by pharmacologically reducing plasma glutamate concentrations, leading to a potentiated glutamate transfer from the brain to blood. This glutamate scavenging mechanism could be the basis of a novel therapeutic strategy to decrease glutamate-induced toxicity in the brain [5, 90].

4.2.3 The KYN Pathway

Inflammatory activation leads to the release of several inflammatory cytokines such as IFN-gamma and TNF that stimulates IDO, which in turn leads to the generation of a series of neuroactive and oxidative stress-promoting metabolites that not only lead to widespread behavioral changes but also yield several metabolites that influence glutamate neurotransmission. The impact of KYN pathway on the etiology of psychiatric disorders have been covered extensively elsewhere [77, 116–118], and only data relevant to the impact of KYN metabolites on glutamate will be presented here. Once activated by IDO, tryptophan is converted into KYN, which is then transported into the brain through the large amino acid transporter in the BBB [119]. Transported KYN is absorbed and processed differentially by astrocytes and microglia.

In astrocytes, KYN is metabolized by kynurenine aminotransferase (KAT) II and converted into kynurenic acid (KYNA), which acts as an NMDA antagonist at its glycine site and is believed to possess putative neuroprotective properties [120]. This hypothesis needs to be balanced against its robust antagonist effects on alpha 7 nicotinic acetyl choline receptors (alpha 7-nAChR) and decreased pathological and physiological presynaptic glutamate release, both of which have been implicated in the etiology of schizophrenia [116, 120]. In microglia, KYN is sequentially converted into 3-hydroxy kynurenine (3HK), anthranilic acid (AA), and 3-hydroxy anthranilic acid (HANA), which are profound generators of oxidative stress that increase demand for GSH further leading to glutamate release through system Xc- transporters [5]. Following the above three metabolites, this pathway proceeds to generate quinolinic acid (QUIN) and finally culminates in the formation of nicotinic acid adenine dinucleotide (NAD) – a key reducing agent that further protects against oxidative stress [116]. QUIN is a powerful NMDA receptor agonist and exerts profound neurotoxicity through sustained receptor stimulation [120, 121]. QUIN results in increased presynaptic (through activation of presynaptic NMDA receptors) and astrocytic glutamate release (gliotoxic effects) as well as oxidative stress, and the toxic effects of increased glutamate on postsynaptic NMDA receptors is exaggerated by QUIN [120, 121]. Thus, sustained presence of QUIN even at physiological concentrations can lead to toxic neural and glial activity, and consequently the synthesis and metabolism of QUIN is a key target for drug development for a variety of disorders [122]. Behaviorally, increased QUIN has been associated with behavioral symptoms of depression and suicidality [123–125] as well as structural brain changes in brain regions that regulate mood and cognition such as the striatum, hippocampus, and prefrontal cortex [126–129].

5 Preclinical and Clinical Studies

Given the relatively new consideration that inflammation may exert its behavioral effects in part through affecting glutamate neurotransmission, there have been few laboratory animal studies that have examined whether blocking glutamate receptors

might reverse inflammation-induced depressive-like behavior. The study that most directly addresses this question involved the use of the NMDA receptor antagonist ketamine to prevent the development of LPS-induced depressive-like behavior in mice [56]. In this study, animals were pretreated with ketamine and then immediately administered with LPS. Ketamine treatment had no effect on increased inflammatory cytokine expression, activation of IDO, or the expression of BDNF in the brain. Nevertheless, ketamine completely reversed immobility in the forced swim test and decreased sucrose preference, a measurement of anhedonia. Establishing the receptor specificity of these effects, inhibition of AMPA receptors, which have been shown to mediate the antidepressant effects of ketamine, restored LPS-induced decreases in sucrose preference [56]. Consistent with these findings, in a rat model of treatment-resistant depression using chronic administration of ACTH, response to ketamine was found to be associated with increased peripheral blood inflammatory markers including plasma CRP and TNF [130]. Similar results were found in a small clinical study in which peripheral blood concentrations of IL-1-beta and IL-6 were found to be elevated at baseline (before ketamine administration) in treatment-resistant depressed patients who responded to ketamine versus those who did not [131]. Increased BMI and adipokines (both associated with an inflammatory state) have also been shown to predict treatment response to ketamine [132]. Taken together, these data suggest that drugs that block glutamate receptors may have special efficacy in MDD patients with increased inflammation and increased inflammatory biomarkers may predict which MDD patients are most likely to respond to NMDA or other glutamate receptor antagonists.

To further evaluate the relationship between inflammation and glutamate metabolism in the CNS, work from our group has used MRS to focus on patients undergoing treatment with the inflammatory cytokine IFN-alpha as well as patients with MDD. In our first study, we assessed glutamate normalized to creatine using single voxel MRS in otherwise non-depressed patients with hepatitis C virus (HCV) undergoing treatment with IFN-alpha. Voxels were located in the right and left basal ganglia as well as the dorsal anterior cingulate cortex (dACC) (Fig. 2) [133]. These brain regions were chosen based on an extensive literature using positron emission tomography and functional magnetic resonance imaging to demonstrate reliable engagement of basal ganglia and dACC circuits following administration of inflammatory stimuli [26, 134–138]. Patients were studied before and following 4 weeks of IFN-alpha administration. A control group of HCV-infected patients awaiting IFN-alpha treatment were studied in parallel. IFN-alpha administration was associated with significant increases in glutamate (Glu) normalized to creatine (Cr) in the single voxel measurements taken from the left basal ganglia and the dACC. In addition, increases in the left basal ganglia Glu/Cr ratio were correlated with reduced motivation, and increases in the dACC Glu/Cr ratio were associated with depressive symptom severity. Given the exaggerated CNS inflammatory responses that have been well characterized among aged laboratory animals, a post hoc analysis of our IFN-alpha study was conducted taking age into consideration [139]. Consistent with laboratory animal studies, it was observed that the largest effect of IFN-alpha on Glu/Cr in the left basal ganglia

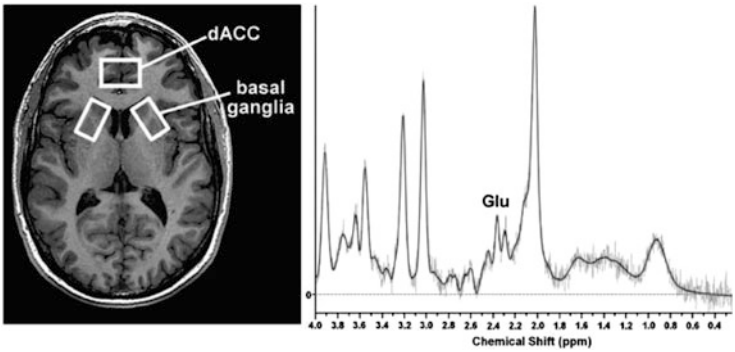

Fig. 2 Placement of MRS voxels and representative spectrum. Magnetic resonance spectroscopy (MRS) voxels were placed as indicated: A $2 \times 3 \times 1$ cm^3 voxel in the dorsal anterior cingulate region (dACC)(BA24) and two $1.7 \times 3 \times 1.7$ cm^3 voxels in the left and right basal ganglia. Glu, glutamate. Reprinted by permission [133]

was seen in individuals greater than 55 years old [140]. Moreover, increased Glu/Cr in the left basal ganglia in older but not younger IFN-alpha-treated and untreated HCV-infected patients was correlated with increased TNF and reduced motivation as well as decreased psychomotor speed as measured by the choice movement time on the Cambridge Neuropsychological Test Automated Battery (CANTAB). Taken together, these data support the notion that inflammation can cause increased normalized glutamate concentrations in CNS regions relevant to the effects of cytokines on the brain and that these effects are most prominent as a function of increasing age.

To extend these studies, the relationship between inflammation and glutamate metabolism as measured by MRS was examined in patients with MDD [12]. Both single voxel MRS and chemical shift imaging (CSI), an MRS technique that measures CNS metabolites over multiple small voxels, were employed. Similar to the findings in patients receiving IFN-alpha, increased inflammation as reflected by plasma CRP was associated with a stepwise increase in absolute glutamate concentrations in the left basal ganglia as measured by single voxel MRS (Fig. 3). A linear relationship between increased plasma CRP and left basal ganglia glutamate was also observed. In addition, increased left basal ganglia glutamate was significantly correlated with anhedonia and psychomotor slowing as measured by the finger-tapping task, simple reaction time task of the CANTAB, and the digit symbol substitution task (all measures of psychomotor speed) (Fig. 4). All findings were significant after controlling for relevant covariates including age, sex, race, body mass index, smoking, and depression severity. Importantly, these latter results also remained significant after controlling for CRP, indicating that any relationship between CRP and behavior was being mediated by the relationship between CRP (a proxy for inflammation) and glutamate metabolism. Studies using CSI gave similar results, with increased plasma and CSF CRP being significantly correlated with left basal ganglia glutamate normalized to creatine [12]. Of the metabolites

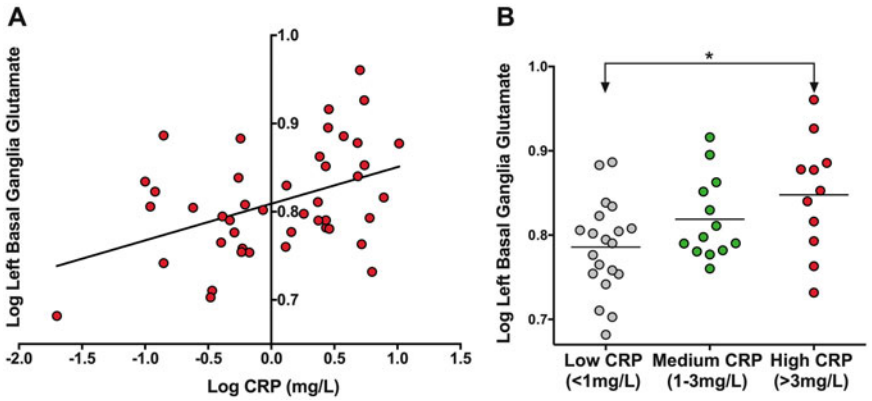

Fig. 3 Association between plasma C-reactive protein (CRP) and the left basal ganglia glutamate in depressed patients. Plasma CRP was positively associated with the left basal ganglia absolute glutamate in both a linear (**a**) and stepwise manner (**b**) in medication-free patients with major depressive disorder. (**a**) Increased log plasma CRP correlated and log left basal ganglia absolute glutamate concentrations in depressed subjects after controlling for age, sex, race, smoking status, body mass index, and 17-item Hamilton Depression Rating Scale scores ($\beta = 0.36$, $t = 2.57$, $P = 0.014$). (**b**) Groupwise comparison of log left basal ganglia absolute glutamate concentrations among patients grouped by CRP revealed a significant main effect of group ($F[2,43] = 4.42$, $P = 0.018$). *$P < 0.025$. Reprinted by permission [12]

measured by CSI other than glutamate, only myoinositol was significantly correlated with left basal ganglia glutamate normalized to creatine. These data are intriguing given that myoinositol is believed to be a marker of astrocyte function and in this case potentially astrocyte dysfunction. One other interesting feature of the CSI studies was the ability to compare results from the study on IFN-alpha-treated patients and MDD subjects, given that both measured glutamate normalized to creatine [12]. Interestingly, control subjects in the IFN-alpha study exhibited Glu/Cr concentrations almost identical to MDD patients with a CRP less than 1 mg/L. Moreover, the increase in Glu/Cr concentrations in MDD patients with high inflammation (CRP >3 mg/L) was about half the increase seen in IFN-alpha-treated patients. These data not only demonstrate consistency of glutamate measures across qualitatively different study populations but also suggest that there may be a dose response relationship between increased inflammation and increased basal ganglia glutamate such that more potent, exogenously administered inflammatory stimuli (e.g., IFN-alpha) lead to greater increases in CNS glutamate, whereas elevations in inflammation from endogenous sources lead to somewhat lesser increases.

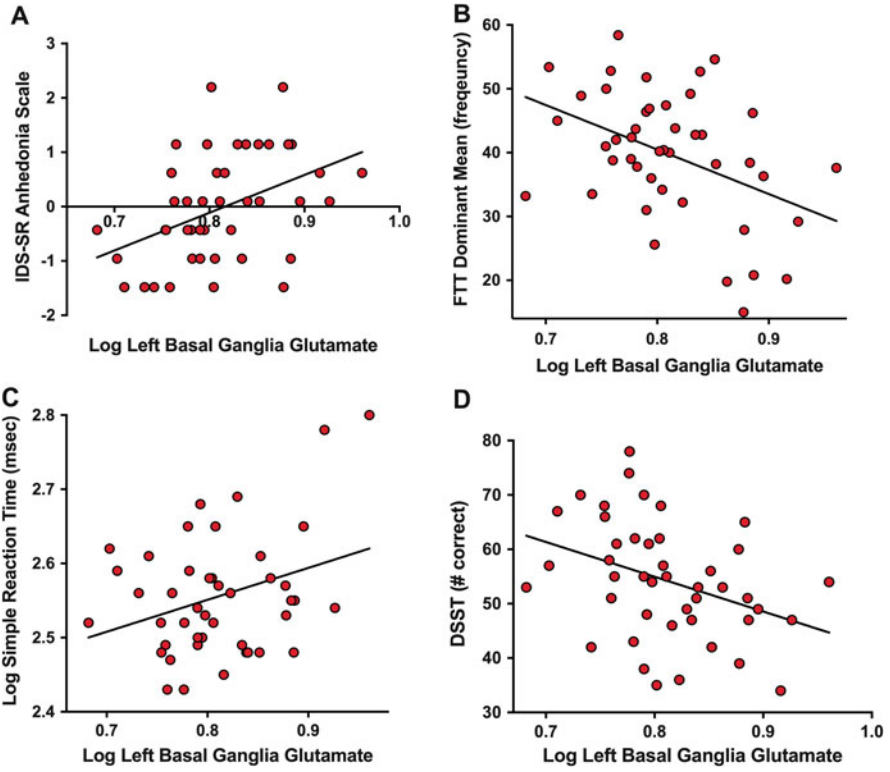

Fig. 4 Correlations between the left basal ganglia glutamate and anhedonia and psychomotor speed in depressed patients. (**a**) Logged values of absolute glutamate concentrations in the left basal ganglia were correlated with the IDS-SR anhedonia subscale ($\beta = 0.42$, $t = 3.03$, $P = 0.004$). (**b**) Log absolute basal ganglia glutamate concentrations were correlated with finger-tapping frequency on the FTT ($\beta = -0.40$, $t = -2.96$, $P = 0.005$). (**c**) Log absolute basal ganglia glutamate concentrations were correlated with log simple reaction time assessed using the CANTAB ($\beta = 0.35$, $t = 2.44$, $P = 0.019$). (**d**) Log absolute basal ganglia glutamate concentrations were correlated with performance on the DSST ($\beta = -0.36$, $t = -2.57$, $P = 0.01$). Statistical tests of significance after controlling for covariates including age, sex, race, body mass index, smoking status, Hamilton Scale of Depression scores, and plasma CRP are indicated in *parentheses*. *CANTAB* Cambridge automated neuropsychological test battery, *DSST* digit symbol substitution test, *FTT* finger-tapping test, *IDS-SR* inventory for depressive symptoms-self rated. Reprinted by permission [12]

6 Treatment Implications

There are several important conclusions that can be derived from these studies on IFN-alpha and MDD subjects as well as the studies in treatment-resistant depression in both clinical and preclinical models. First, increased peripheral inflammatory markers appear to identify patient populations, especially patients with MDD that exhibit increased CNS glutamate, particularly in the basal ganglia

[12]. Moreover, preliminary evidence from laboratory animals and humans suggest that increased inflammatory markers may predict the response to glutamate antagonists (e.g., ketamine) [130–132]. These data suggest that future studies examining the efficacy of glutamate receptor antagonists should focus on patients with increased peripheral inflammatory markers. Second, preclinical studies examining the mechanisms of increased glutamate secondary to inflammation will provide a better understanding of which treatment options are most likely to be efficacious in future studies. Indeed, increased glutamate as measured by an MRS provides no information on whether the glutamate is extracellular or intracellular, although future studies using 13C-MRS in patients with varying levels of inflammation may provide important insight. Third, studies examining CNS glutamate using MRS can be coupled with magnetic resonance imaging to evaluate the relationship between white matter (glia) integrity as measured by diffusion tensor imaging and glutamate. Based on the impact of glutamate-mediated excitotoxicity on astrocytes and oligodendrocytes, one might expect that glutamate increases would be associated with decreased white matter integrity as measured by fractional anisotropy and other techniques. Longitudinal studies tracking parallel changes in these measures over time may further identify patients at risk for neurodegeneration, cognitive dysfunction, and potentially dementia, all associated with MDD. Finally, further link between inflammation and alterations in CNS glutamate neurotransmission should be established through the use of specific anti-inflammatory agents. Such data will open up the possibility of using anti-inflammatory agents to target alterations in glutamate metabolism with the hope that CNS glutamate can serve as evidence of target engagement in the brain and as a proximal endpoint for a potentially successful therapeutic intervention in the course of drug development. These studies would also lay the groundwork for potentially combining glutamate and inflammation-targeted therapies for treatment and prevention of relevant neuropsychiatric disorders including depression.

7 Summary and Conclusions

Taken together, there is a strong foundation for the hypothesis that inflammation may mediate its effects on behavior especially depression through affecting glutamate neurotransmission. These effects appear to find a cellular basis in the impact of inflammation on astrocytes and microglia as well as oligodendrocytes, while effects on glutamate reuptake and release and gliotransmission are targets of inflammatory cytokines and their signaling pathways on a molecular and metabolic level. This convergence of pathology between inflammation and glutamate opens up a host of therapeutic options ranging from inhibiting inflammation to reverse glutamate- and glia-related pathologies to inhibiting glutamate to resolve the effects of inflammation on behavior. Although there is a rich literature demonstrating the impact of inflammation on the regulation of glutamate and vice versa in laboratory animal models and neuropathology, there are a paucity of studies examining how

these two systems interact in psychiatric disorders such as depression. Future studies are warranted to further explore the interactions between inflammation and glutamate in depression with the hope of developing novel treatments for those 1/3 of depressed patients who are unable to respond to conventional antidepressant therapies.

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The Role of Dopamine in Inflammation-Associated Depression: Mechanisms and Therapeutic Implications

Jennifer C. Felger

Abstract Studies investigating the impact of a variety of inflammatory stimuli on the brain and behavior have consistently reported evidence that inflammatory cytokines affect the basal ganglia and dopamine to mediate depressive symptoms related to motivation and motor activity. Findings have included inflammation-associated reductions in ventral striatal responses to hedonic reward, decreased dopamine and dopamine metabolites in cerebrospinal fluid, and decreased availability of striatal dopamine, all of which correlate with symptoms of anhedonia, fatigue, and psychomotor retardation. Similar relationships between alterations in dopamine-relevant corticostriatal reward circuitry and symptoms of anhedonia and psychomotor slowing have also been observed in patients with major depression who exhibit increased peripheral cytokines and other inflammatory markers, such as C-reactive protein. Of note, these inflammation-associated depressive symptoms are often difficult to treat in patients with medical illnesses or major depression. Furthermore, a wealth of literature suggests that inflammation can decrease dopamine synthesis, packaging, and release, thus sabotaging or circumventing the efficacy of standard antidepressant treatments. Herein, the mechanisms by which inflammation and cytokines affect dopamine neurotransmission are discussed, which may provide novel insights into treatment of inflammation-related behavioral symptoms that contribute to an inflammatory malaise.

Keywords Anhedonia • Cytokines • Depression • Dopamine • In vivo microdialysis • Inflammation • Motivation • Motor slowing • Neuroimaging • Striatum

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1 Introduction: Inflammation Effects on Dopamine-Relevant Depressive Behaviors

Findings from numerous laboratories have consistently indicated that innate immune activation and the release of inflammatory cytokines preferentially affect reward pathways and brain dopamine to contribute to reduced motivation and motor slowing [1–6]. The effect of inflammatory cytokines on motivation and motor activity may be especially relevant to depressive symptoms of anhedonia, fatigue, and psychomotor retardation, which are commonly observed in patients chronically administered inflammatory cytokines such as interferon (IFN)-α [7, 8]. Furthermore, these inflammation-related symptoms relevant to reduced motivation and motor slowing are difficult to treat with standard antidepressant therapies like selective serotonin reuptake inhibitors (SSRIs) in patients with major depression [9–12], as well as in those treated with IFN-α [7, 8, 13]. Given the longstanding relationship between inflammation and depression [14, 15], particularly in patients who are resistant to antidepressant treatment, these findings suggest that other neurotransmitter systems, such as dopamine, may be involved in these SSRI-resistant, inflammation-related symptoms. Nevertheless, classical stimulant medications that increase dopamine release and/or block dopamine reuptake have demonstrated limited efficacy in the treatment of fatigue in patients with inflammation-associated medical illnesses [16–18]. Therefore, a better understanding of the mechanisms by which inflammation and cytokines may affect dopamine function will inform strategies to improve the treatment of depressive symptoms related to reduced motivation and motor slowing in medically ill and medically healthy individuals.

This chapter will highlight the current literature demonstrating the impact of peripheral inflammation on brain dopamine by integrating findings from both clinical and translational studies involving acute or chronic administration of cytokines or inflammatory stimuli. Knowledge gained from these administration

studies has informed recent work in patients with major depression revealing similar relationships between increased peripheral cytokines and other inflammatory markers, alterations in dopamine-relevant reward circuitry, and reduced motivation and motor function, which will also be presented [19, 20]. In humans, much evidence stems from studies in healthy volunteers acutely administered endotoxin or typhoid vaccination, and from patients chronically administered inflammatory cytokines (e.g., IFN-α) as therapy for some cancers and infectious diseases. Like endotoxin and vaccination, IFN-α administration induces release of the inflammatory cytokines interleukin (IL)-6, IL-1 and tumor necrosis factor (TNF) [21–25]. Depending on the dose, up to 50% of patients administered IFN-α as treatment for hepatitis C virus (HCV) or malignant melanoma meet symptom criteria for major depression, and up to 80% experience significant fatigue, lack of energy, and motor slowing [7, 24, 26–31]. Additionally, reduced motivation and anhedonia are frequently reported in IFN-α-treated patients [2, 6]. Models of administration of cytokines or inflammatory stimuli to humans and laboratory animals will be discussed in the context of potential mechanisms of cytokine effects on dopamine and relevant neurocircuitry. In addition, cytokine effects on other neurotransmitter systems as they relate to dopamine function will be described, and implications of these findings for treatment of inflammation-related depressive symptoms will be discussed.

2 Inflammation Effects on Dopamine and Corticostriatal Neurocircuitry

2.1 Translational Studies

Initial evidence that inflammation can affect brain dopamine originates from neurochemical and behavioral studies in rodents administered acute or sub-chronic IFN-α that reported either increases or decreases in dopamine and/or dopamine metabolites in concert with depressive behaviors and changes in locomotor activity [32–36]. These mixed results were likely due to differences in dosing, length of exposure, and most importantly, lack of use of species-specific IFN-α [37–39]. However, rhesus monkeys exposed to chronic IFN-α exhibit immune, neuroendocrine, and behavioral response similar to that of cytokine-treated patients, including decreases in psychomotor activity and increases in depressive-like huddling behavior (in ~50% of animals) [1, 23]. Of note, depressive huddling behavior in non-human primates was previously described following chronic administration of the monoamine-depleting agent reserpine, and dopamine receptor antagonists and partial agonists [40, 41]. Only animals that displayed depressive behavior following IFN-α administration were found to have significantly lower cerebrospinal fluid (CSF) concentrations of the dopamine metabolites

homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), which correlated with decreased locomotor activity [1, 23].

To further explore the effects of inflammatory cytokines on synaptic availability and release of striatal dopamine, in vivo microdialysis and positron emission tomography (PET) neuroimaging with [^{11}C]raclopride displacement following amphetamine (AMPH) challenge were conducted on IFN-α-treated monkeys [42]. Results indicated that stimulated dopamine release was indeed decreased in the striatum (including nucleus accumbens) after chronic administration of IFN-α, and decreased dopamine release, as measured by in vivo microdialysis, was correlated with reduced effort-based but not freely available sucrose consumption [42]. Furthermore, IFN-α-induced decreases in striatal dopamine release were reversed by the dopamine precursor levodopa (L-DOPA) administered via reverse in vivo microdialysis, indicating that cytokines may reduce dopamine synthesis and availability [43].

Similar to the effects of IFN-α, peripheral administration of interleukin (IL)-1β has been shown to decrease effort-based responses for sucrose reward over freely available chow, in the absence of a decrease in preference of freely available sucrose over chow [44]. Furthermore, a similar decrease in motivation for but not sensitivity to sucrose reward has been reported in mice following lipopolysaccharide (LPS) administration [45]. In terms of effects on dopamine, whereas acute systemic administration of low dose LPS (~100 μg/kg) has been reported to either decrease tissue dopamine content or increase extracellular dopamine metabolites in the nucleus accumbens [46, 47], single injections of septic doses of LPS (5 mg/kg) cause progressive neurodegeneration of the nigrostriatal dopaminergic system [48, 49]. Both the short- and long-term effects of LPS on brain dopamine can be blocked by inhibition or genetic deletion of inflammatory cytokines such as TNF [47, 48, 50]. Finally, models of inflammation-related medical illness, such as experimental tumors, are associated with decreased brain dopamine [51, 52]. Together, these results from animal studies indicate that a variety of inflammatory stimuli have been consistently found to affect brain dopamine to lead to relevant depressive symptoms, and have prompted further investigation into inflammation effects on dopamine and the basal ganglia in clinical populations.

2.2 Human Neuroimaging Studies

Neuroimaging studies across several laboratories suggest that disruption of the basal ganglia and dopamine is a major contributor to inflammation-induced behavioral change. The basal ganglia are key subcortical structures that regulate motivation and motor activity, and dopamine plays an essential modulatory role [53]. In the first study to examine IFN-α effects on the brain, increased glucose metabolism was found in the basal ganglia and particularly the dopamine-rich putamen [54], as assessed by PET with fluorine-18-labeled-fluorodeoxyglucose (FDG). More recently, FDG PET revealed increased basal ganglia glucose metabolism in patients

receiving high dose IFN-α as therapy for malignant melanoma [3]. Increased glucose metabolism in the left putamen and left nucleus accumbens correlated significantly with reports of fatigue in these patients, as assessed by the "energy" subscale of the Visual Analog Scale of Fatigue (VAS-F) [3]. This pattern of increased glucose metabolism in basal ganglia nuclei is similar to that seen in patients with Parkinson's disease (PD) [55–57], where it is thought to reflect increased oscillatory burst activity in relevant basal ganglia nuclei secondary to loss of inhibitory nigral dopamine input [58, 59].

Functional magnetic resonance imaging (fMRI) has also demonstrated decreased neural activation in the basal ganglia, including ventral striatum, to hedonic reward (a gambling task) in HCV+ patients undergoing IFN-α administration, which correlated with self-reported reduced motivation [2]. Administration of the cytokine-inducers LPS and typhoid vaccination to healthy volunteers produces similar effects on the ventral striatum, suggesting that findings from IFN-α generalize to other inflammatory stimuli [4, 5, 60]. Indeed, LPS administration led to reduced activation in the ventral striatum during a monetary reward task that was associated with increases in self-reported depressed mood, as measured by the Profile of Mood States (POMS) depression subscale [5]. Typhoid vaccination, which produces a mild systemic inflammation characterized by increases in circulating IL-6, was shown to cause a shift in reward versus punishment sensitivity in a probabilistic instrumental learning task combined with fMRI [61]. Compared to saline control, vaccination reduced behavioral attractiveness of rewards while making punishments more aversive, effects that were related to neural representations of reward and punishment prediction errors in the ventral striatum as well as anterior insula [61]. Of relevance to potential effects of inflammation on dopamine, the magnitude of response to reward prediction error is fundamentally modulated by dopamine-dependent striatal activity, as determined by administration of drugs that enhance (L-DOPA) or inhibit (haloperidol) dopaminergic function [62]. Additionally, typhoid vaccination compared to saline has been shown to affect activity in the substantia nigra, including decreased activation in response to novelty and increased activation in response to visual stimuli, which was associated with both psychomotor slowing and increased peripheral blood concentrations of IL-6 [4, 60].

To further examine the role of dopamine in the effects of inflammation on basal ganglia metabolism and activation in humans, a PET study was conducted in HCV+, IFN-α-treated subjects using [^{18}F]fluorodopa (FDOPA). Like the dopamine precursor L-DOPA, FDOPA is taken up by dopaminergic neurons and converted by dopamine decarboxylase to dopamine, whereupon it is stored in vesicles for release. Interestingly, both increased uptake and decreased turnover of FDOPA in the caudate, putamen, and ventral striatum of IFN-α-treated patients were found [2]. Baseline and percent change in FDOPA uptake was in turn correlated with IFN-α-induced behavioral alterations including depression and fatigue, as measured by the Montgomery Asberg Depression Rating Scale (MADRS) and Multidimensional-Fatigue-Inventory (MFI), respectively [2]. Increased uptake and decreased turnover of FDOPA in the basal ganglia following IFN-α administration are in stark contrast to that observed in patients with PD where decreased

uptake and increased FDOPA turnover are seen. Decreased uptake of FDOPA in PD is believed to be a function of loss of dopaminergic neurons and/or their projections throughout the basal ganglia [63–65], and intact or increased turnover suggests that the surviving neurons are capable of normal release [64, 66]. Increased FDOPA uptake during IFN-α treatment suggests a potential depletion of dopamine and increased synthetic capacity, which is consistent with decreased striatal dopamine release in IFN-α-treated monkeys that was reversed by L-DOPA, as described above [42, 43].

Despite the abundance of reports indicating changes in basal ganglia and dopamine function in subjects administered cytokines and inflammatory stimuli, little work has been done to investigate similar effects of inflammation in patients who exhibit high inflammation as a function of medical and neuropsychiatric illnesses. One study in patients with chronic fatigue syndrome, who have been frequently reported to exhibit elevated inflammatory markers including cytokines, reported decreased activation of basal ganglia structures, such as caudate and globus pallidus, in response to hedonic reward using the gambling task mentioned above [67]. In patients with depression, many of whom also have high inflammation, a relationship was observed between increased inflammation and decreased functional connectivity within reward-related corticostriatal neurocircuitry [20]. Indeed, increased inflammation (plasma concentrations of CRP as well as cytokines and their soluble receptors) was associated with decreased magnitude of functional connectivity between both the ventral and dorsal striatum and the ventromedial prefrontal cortex (vmPFC), which correlated with symptoms of anhedonia and psychomotor slowing, respectively, symptoms which are prominent in depressed patients with high inflammation [19, 20, 68]. Like the ventral striatum, vmPFC is part of classic reward circuitry that receives significant mesocorticolimbic dopamine innervation [69, 70]. Accordingly, inflammation-related decreases in corticostriatal connectivity within reward circuitry in depression may involve cytokine-induced decreases in dopamine, and have potential for reversal with pharmacological strategies that increase dopamine availability or receptor signaling [1].

Together these data from humans and laboratory animals indicate that inflammation-related decreases in dopamine availability and release may have functional consequences on reward circuitry that are associated with fundamental alterations in motivation and motor function. This work supports further consideration of the mechanisms of cytokine effects on dopamine synthesis, release, reuptake, or receptor signaling, which may lead to the development of novel therapeutic strategies to increase dopamine availability in patients with increased inflammation.

3 Mechanisms of Inflammation Effects on Dopamine Neurotransmission

Cytokines can potentially affect multiple aspects of dopamine neurotransmission, leading to decreased synthesis, impaired packaging and release, or increased reuptake, all of which may interact to a greater or lesser extent to reduce dopamine neurotransmission in the basal ganglia (see Fig. 1). The following section will discuss potential mechanisms by which cytokines affect these aspects of dopamine function, ultimately resulting in reduced dopamine signaling.

3.1 Dopamine Synthesis and Availability

Dopamine synthesis relies on the conversion of tyrosine to L-DOPA by tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine synthesis. A major source of tyrosine is phenylalanine, which is converted to tyrosine by phenylalanine hydroxylase (PAH). Both of these enzymes, TH and PAH, require the enzyme cofactor tetrahydrobiopterin (BH4). Although inflammation and cytokines have been shown to induce GTP-cyclohydrolase I, the enzyme necessary for BH4 synthesis, inflammation may in turn decrease BH4 availability [71]. BH4 is also a cofactor for nitric oxide synthases (NOS). Inflammation-induced increases in inducible NOS (iNOS) activity can usurp available BH4, which results in NOS uncoupling and the generation of reactive oxygen species instead of NO [72, 73]. This increase in oxidative stress can then contribute to oxidative reduction of BH4 itself (which is highly redox-sensitive), leaving even less BH4 available for dopamine synthesis (Fig. 1, mechanism 1) [72]. Indeed, intramuscular injection of rats with IFN-α has been shown to decrease CNS concentrations of BH4 through stimulation of NO, and inhibition of NOS was found to reverse IFN-α's inhibitory effects on brain concentrations of both BH4 and DA [32]. Of note, IL-6 treatment has also been shown to reduce BH4 content in sympathetic neurons [74].

Concentrations of phenylalanine, tyrosine, BH4, and BH2 can be measured in the peripheral blood and CSF, and the BH4/BH2 and phenylalanine/tyrosine ratios have been proposed as indicators of BH4 availability and PAH activity, and may serve as indirect biomarkers of dopamine synthetic capacity [71, 75–78]. For example, a number of patient populations with increased inflammation, including patients with trauma, sepsis, cancer, and HIV, have been found to exhibit increased peripheral blood concentrations of phenylalanine [71]. Furthermore, increased phenylalanine concentrations in patients with cancer have been correlated with markers and mediators of inflammation including IL-6, IL-2 receptor, and soluble TNF-α receptor-2, as well as peripheral blood markers of oxidative stress [71]. Moreover, in a study of healthy elderly persons with low-grade inflammation, peripheral blood concentrations of phenylalanine, tyrosine, and an increased

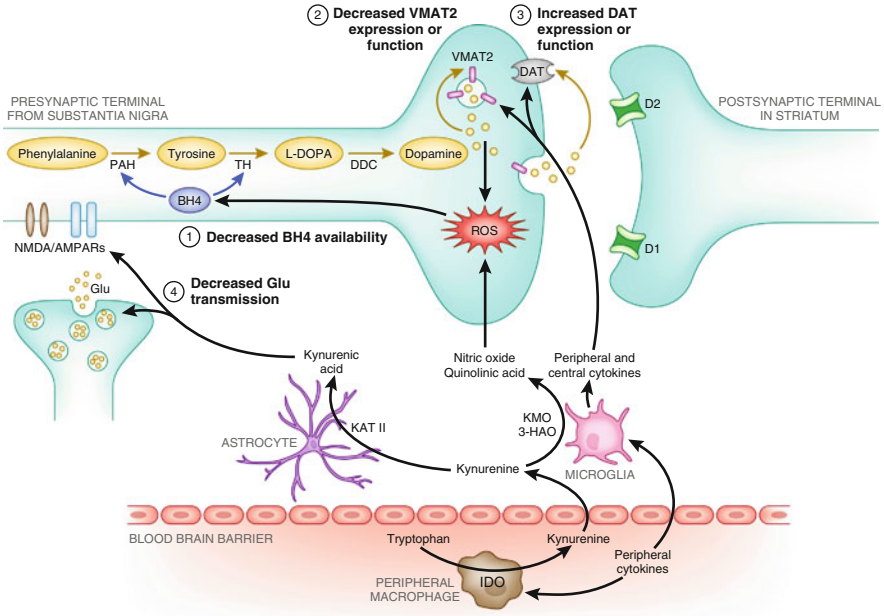

Fig. 1 Potential mechanisms of inflammation effects on dopamine synthesis and release. Evidence indicates that inflammation and release of cytokines from the periphery, or those produced locally by activated microglia or infiltrating macrophages, can produce nitric oxide, as well as quinolinic acid through indoleamine 2,3-dioxygenase (IDO) and kynurenine pathways, both of which contribute to oxidative stress and reactive oxygen species (ROS) generation. Increased ROS and inflammation-induced nitric oxide contribute to (**1**) oxidation of tetrahydrobiopterin (BH4), a cofactor required for the conversion of phenylalanine to tyrosine and tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), which are necessary for the synthesis of dopamine. Furthermore, some evidence exists that inflammatory cytokines may (**2**) decrease the expression or function of the vesicular monoamine transporter 2 (VMAT2), and/or (**3**) increase expression or function of the dopamine transporter (DAT). Dysregulation of dopamine transport and vesicular packaging mechanisms can increase cytosolic dopamine, leading to auto-oxidation and generation of ROS and neurotoxic quinones. Finally, inflammation-related activation of IDO in peripheral immune cells or microglia also produces kynurenic acid from kynurenine by kynurenine aminotransferase (KAT) II activity in astrocytes. Kynurenic acid can lead to (**4**) reduced glutamate (glu) neurotransmission by antagonism of glu receptors and release, consequently decreasing glu-evoked dopamine release in the striatum. Although not pictured, excessive inflammation-induced release of glutamate and quinolinic acid may also contribute to increased oxidative stress and excitotoxicity. *3-HAO* 3-hydroxyanthranilic acid oxygenase, *AMPAR* 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid receptor, *BH4* tetrahydrobiopterin, *D1* dopamine receptor 1, *D2* dopamine receptor 2, *DAT* dopamine transporter, *glu* glutamate, *DDC* dopamine decarboxylase, *IDO* indoleamine 2,3 dioxygenase, *KAT II* kynurenine aminotransferase II, *KMO* kynurenine 3-monooxygenase, *L-DOPA* L-3,4-dihydroxyphenylalanine, *NMDAR* *N*-Methyl-D-aspartic acid receptor, *PAH* phenylalanine hydroxylase, *ROS* reactive oxygen species, *TH* tyrosine hydroxylase, and *VMAT2* vesicular monoamine transporter 2. Reproduced with permission, Felger and Miller [1]

phenylalanine/tyrosine ratio were associated with neuropsychiatric symptoms including anhedonia and altered sleep [75].

Evidence of reduced BH4 activity has also been observed in IFN-α-treated patients [79, 80]. For example, IFN-α administration was associated with increased peripheral blood phenylalanine/tyrosine ratio, which in turn correlated with decreased CSF dopamine and its major metabolite HVA [79]. Increased cerebrospinal fluid (CSF) IL-6 was also correlated with decreased BH4 in CSF of IFN-α-treated patients, and the phenylalanine/tyrosine ratio significantly correlated with IFN-α-induced depressive symptoms [79]. These findings are consistent with decreased dopamine metabolites in the CSF of both IFN-α-treated patients and monkeys [1, 23], and with the complete reversal of IFN-α-induced decreases in dopamine by L-DOPA administered via reverse in vivo microdialysis in monkeys [81]. Of note, during L-DOPA administration, no changes were found in the DOPAC/dopamine ratio, which increases when dopamine is not properly packaged in synaptic vesicles and is subsequently metabolized via monoamine oxidase [82]. Although these findings strongly suggest that inflammatory cytokines reduce dopamine availability through a deficiency in its precursor, without effects on end-product synthesis or vesicular packaging and release, some evidence exists indicating that cytokines may also target dopamine packaging, release, and reuptake mechanisms, as presented below.

3.2 Dopamine Packaging, Release, and Reuptake

Synaptic dopamine is dependent on the vesicular monoamine transporter 2 (VMAT2) to package cytosolic dopamine into vesicles for release. There is some evidence that inflammatory cytokines and inflammation may negatively affect the expression and function of VMAT2 (Fig. 1, mechanism 2). For example, the inflammatory cytokines IL-1 and TNF were found to decrease expression of VMAT2 in rat enterochromaffin-like cells, whereas transforming growth factor-beta, which is immunomodulatory and anti-inflammatory, increased VMAT2 expression [83]. Additionally, the anti-inflammatory compound, pituitary adenylate cyclase-activating polypeptides 38, administered in vivo by subcutaneous minipump, was able to increase VMAT2 expression, reduce neuroinflammation and oxidative stress, and protect against dopamine neurotoxicity following chronic methamphetamine exposure [84].

Attention has been paid to the effects of cytokines and inflammatory signaling pathways on monoamine reuptake pumps, and particularly the serotonin transporter [85–88]. Both in vitro and in vivo data have established that stimulation of p38 mitogen-activated protein kinase (MAPK), a major signaling pathway activated by IFN-α and other cytokines, can increase the expression and function of the serotonin transporter, leading to increased serotonin reuptake [86–88]. MAPK pathways have also been found to influence the dopamine transporter (DAT). For example, DAT-expressing cells transfected with a constitutively activate MAPK kinase

(MEK) show increased DA reuptake (Vmax), whereas treatment of rat striatal synaptosomes with MEK inhibitors was associated with decreased DA reuptake in a concentration and time-dependent manner [85]. Furthermore, subjects with neuropsychiatric disturbances as a result of HIV infection and subsequent neuroinflammation are thought to have increased expression of DAT [89, 90]. Therefore, reduced dopamine turnover secondary to inflammatory cytokines may be mediated, in part, by increased DAT expression or function (Fig. 1, mechanism 3). However, no change in DAT binding, as measured by PET with 18F-labeled FECNT, was observed in monkeys exposed to chronic IFN-α [42].

3.3 Glutamate Neurotransmission and Dopamine Release

Another mechanism by which cytokines may influence the basal ganglia and dopamine function is through effects on glutamate neurotransmission. For example, there has been recent interest in the impact of cytokine stimulation of indoleamine 2,3 dioxygenase (IDO) and downstream kynurenine pathway metabolites on glutamate neurotransmission in the brain [91, 92]. Immune-mediated activation of IDO catabolizes tryptophan, the primary amino-acid precursor of serotonin, to kynurenine. Kynurenine is further catabolized into the neuroactive metabolites kynurenic acid (KA) (in astrocytes) and quinolinic acid (QUIN) (in microglia), both of which have been found to be increased in the plasma and CSF of IFN-α-treated patients [27, 93–95]. Of note, CSF QUIN significantly correlated with depressive symptoms during IFN-α administration, as measured by MADRS [94]. In addition to increasing oxidative stress [96, 97], the neurotoxic metabolite QUIN can also directly activate the *N*-methyl-D-aspartate (NMDA) receptor to induce the release of glutamate to lead excitotoxicity in the brain [93, 98, 99], thus further increasing inflammation and its potential effects on the dopamine system described above [100, 101]. In contrast to QUIN, KA reduces glutamate release, and has been shown to be an antagonist of NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [102]. Dopamine release is in part under glutamatergic control, and thereby KA may exert downstream effects on DA (Fig. 1, mechanism 4) [93]. Indeed, intra-striatal administration of KA to rodents leads to marked reductions in extracellular dopamine concentrations, as determined by in vivo microdialysis [103].

Finally, cytokines and inflammation have been shown to increase glutamate by effects on microglia and astrocytes. A rich literature has shown that cytokines can decrease the astrocytic expression of glutamate transporters, and increase release of glutamate from astrocytes and activated microglia in vitro [91, 104–106]. Of note, glutamate released from glia may have preferential access to extrasynaptic NMDA receptors, which lead to decreased production of trophic factors including brain-derived neurotrophic factor [107, 108]. Given the sensitivity of dopamine neurons to oxidative stress and excitotoxicity, inflammation effects on glutamate may

contribute to decreased dopamine availability and eventual neurodegeneration [109, 110].

4 Translational Implications: Potential Therapeutic Targets for Inflammation Effects on Dopamine

The data summarized herein demonstrate that inflammatory cytokines affect dopamine function and may contribute to the development of depressive symptoms relevant to reduced motivation and motor activity in multiple patient populations with increased inflammation. Current antidepressant therapies are effective for many patients with major depression. However, up to 30% fail to achieve remission and even responders often exhibit significant residual symptoms that are consistent with those that are caused by exposure to cytokines and inflammation, such as anhedonia, fatigue, and psychomotor retardation [9–12]. Non-responsiveness of inflammation-related symptoms to standard antidepressant therapies has been exemplified in patients receiving IFN-α therapy who were treated with SSRIs. SSRIs alleviated IFN-α-induced anxiety and some depressive symptoms, but not those of fatigue or psychomotor retardation [7, 8, 13]. Additionally patients with advanced cancer undergoing chemotherapy exhibit increased inflammation in association with fatigue that is not responsive to SSRIs [111–113]. Therefore, new conceptual frameworks are needed to treat these inflammation-associated symptoms [114–116], which may respond to novel treatment strategies that target the dopamine system.

As discussed previously, classical stimulant medications that increase dopamine release and/or block dopamine reuptake have demonstrated only limited efficacy in the treatment of fatigue in patients with cancer and other medical illnesses associated with inflammation [16–18, 117–120]. Since stimulants act to increase dopamine release and block DAT function, these drugs may not provide long-term efficacy if cytokine effects on dopamine function are primarily mediated through inhibitory effects on synthesis, packaging, or release. Therefore, consideration should be given to alternative strategies such as compounds that increase dopamine synthesis, packaging, or receptor signaling, or those that inhibit activation of the neuroactive metabolites of the kynurenine pathway and/or glutamate. Of course, strategies that inhibit inflammation and/or the inflammatory cytokines themselves may also be considered. Indeed, inhibition of inflammatory cytokines, such as TNF, has been shown to reduce depressive symptoms including anhedonia and psychomotor slowing in patients with inflammatory disorders and in depressed patients with increased inflammation [121–124].

Considering the strong evidence presented above indicating that inflammation can inhibit key components of dopamine synthesis, pharmacologic strategies that increase dopamine may effectively treat inflammation-related symptoms of anhedonia, fatigue, and psychomotor slowing. For instance, there are a number of

compounds that can boost BH4 availability or activity, which may facilitate the capacity of PAH and TH to synthesize dopamine. These include administration of BH4 itself [125], which is currently approved in a synthetic form to treat phenylketonuria [126–128], as well as folic acid, L-methylfolate, or S-adenosyl-methionine (SAMe), all of which have a role in the synthesis and/or regeneration of BH4 [1, 129, 130] and have demonstrated efficacy as adjuvants to antidepressants [131–133]. Although effects of BH4 administration on depressive and motor symptoms have not been reported outside of case reports [134, 135], trials examining the efficacy of folic acid, L-methylfolate, and SAMe have been conducted in depression. Interestingly, low serum folate has been associated with increased risk of depression, as well as non-response to antidepressant treatment and an increased likelihood of depression relapse [136–140]. Administration of L-methylfolate (marketed as Deplin and Zervalx) to depressed patients has been shown to augment the efficacy of standard antidepressant therapy [132, 133], and treatment with SAMe adjunctive to SSRIs leads to significantly higher rates of remission and 50% or greater decreases in depressive symptoms compared with placebo [131]. Therefore, strategies to augment BH4 activity exhibit potential efficacy for restoring dopamine function and treating fatigue and depression in patients with increased inflammation.

In terms of targeting dopamine packaging and release, compounds that improve VMAT2 function could be considered for the treatment of cytokine-induced depression and fatigue. For instance, VMAT2 activity can be increased with trkB agonists, and the small molecule trkB agonist 7,8-dihydroxyflavone was neuroprotective in a rodent model of Parkinson's disease [141]. Adenosine receptor antagonists, which are thought to facilitate activation of dopamine D2 receptors, also reversed peripheral cytokine-induced decreases in effort-based sucrose consumption in rats [44]. Finally, inhibition of the IDO pathway or glutamate may be an important target in addressing the impact of inflammation on basal ganglia dopamine function and treating inflammation-induced depression and fatigue. The IDO antagonist, 1-methyl tryptophan (1-MT) has been shown to abrogate the impact of LPS, as well as an attenuated form of *Mycobacterium bovis*, on depressive-like behavior [142, 143]. As mentioned above, dopamine release is under partial control of glutamate neurotransmission, and changes in dopamine function due to inflammation-induced KA may respond to α 7 nicotinic acetylcholine receptor agonists, that have been shown to reverse kynurenic acid effects on dopamine release [103]. Administration of glutamate receptor antagonists, such as the NMDA antagonist, ketamine, has potent antidepressant effects especially in treatment resistant depressed patients who have been shown to exhibit increased inflammation [144, 145]. Given that the neurotoxic effects of QUIN may be mediated by excessive glutamate excitotoxicity [93, 98, 99], glutamate antagonists may be useful in preventing excitotoxic effects on the highly sensitive dopamine neurons. Indeed, metabotropic glutamate receptor antagonists that modulate glutamate transmission in the basal ganglia have been successful in reducing dopamine cell loss in an animal model of PD [146], and antagonism of the NMDA receptor with memantine also reversed loss of dopamine content in the striatum of

monkeys infected with simian immunodeficiency virus (SIV) [147]. Therefore, blockade of kynurenine pathways or modulation of glutamate neurotransmission may confer protection against inflammation and IDO-mediated effects on dopamine function, to improve depressive symptoms in patients with increased inflammation.

5 Summary and Conclusions

There is strong evidence that inflammatory cytokines specifically target dopamine and reward circuitry to contribute to depressive symptoms relevant to reduced motivation and motor slowing. Much of this evidence stems from biochemical and behavioral studies in humans and animals administered inflammatory stimuli, and from neuroimaging experiments demonstrating altered basal ganglia and dopamine function in association with increased peripheral immune activation and depressive symptoms. These inflammation-related depressive symptoms, such as anhedonia, fatigue, and psychomotor retardation, are resistant to treatment with SSRIs in patients with medical illnesses and/or major depression. Surprisingly, these symptoms have also been difficult to treat with classical stimulant medications that increase DAT-mediated dopamine release and/or block dopamine reuptake, indicating cytokine effects on dopamine function may sabotage or circumvent the mechanism of action of these agents. Evidence suggests that inflammatory cytokines may affect multiple aspects of dopamine neurotransmission, leading to decreased synthesis and/or impaired packaging or release, all of which may interact to a greater or lesser extent to reduce dopamine function. Multiple potential pharmacological treatment strategies exist, yet future studies are needed to identify precise targets for reversing inflammation effects on brain dopamine. Further understanding of the effects of inflammation and cytokines on dopamine and reward circuitry will guide future development and testing of novel treatment strategies to reverse dopamine-relevant behavioral changes in patients with increased inflammation.

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Brain Structures Implicated in Inflammation-Associated Depression

Neil A. Harrison

Abstract Systemic inflammation rapidly impairs mood, motivation, and cognition inducing a stereotyped cluster of symptoms collectively known as "sickness behaviors." When inflammation is severe or chronic, these behavioral changes can appear indistinguishable from major depressive disorder (MDD). Human and rodent neuroimaging combined with experimental inflammatory challenges has clarified the neural circuitry associated with many of the key features of inflammation-induced-sickness behavior, and in so doing revealed often-remarkable commonalities with circuit abnormalities observed in MDD. This review aims to provide the first synthesis of this work illustrating areas of convergence and divergence with the MDD literature as well as highlighting areas for future study.

Keywords Amygdala • Cytokine • Depression • fMRI • Inflammation • Insula • Interferon • Microglia • MRI • PET • Ventral striatum

Contents

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1 Introduction

Human and animal studies implicate systemic inflammation in the pathogenesis of depression [1]. In healthy mammals, systemic infection triggers profound behavioral changes, including cognitive and mood symptoms (e.g., memory impairment, social withdrawal, anxiety, and depression), change in motivation (anorexia, adipsia, and anhedonia), and neurovegetative symptoms (sleep disturbance, fatigue, and psychomotor slowing) [2–4] known as sickness behaviors. Clinical and pre-clinical studies suggest that inflammatory cytokines play a central role in mediating these sickness-related behaviors by communicating peripheral inflammation to the brain. These cytokine-induced sickness behaviors show striking similarity to symptoms of major depression [1] supporting a role for immune–brain interactions in the etiology of at least some patients with major depressive disorder (MDD).

In rodents, systemic administration of interleukin (IL)-1β or bacterial lipopolysaccharide (LPS), a potent stimulant of cytokine release, can rapidly elicit a depression-like syndrome characterized by a reduction in positively motivated approach behaviors such as exploration, social interaction, and operant behaviors for food reward [5–7]. Similarly, experimental induction of inflammation in healthy human participants using either LPS [8, 9] or typhoid vaccination [10–12] induces symptoms of fatigue, psychomotor slowing, mild cognitive confusion, memory impairment, social withdrawal, anxiety, and deterioration in mood that mirror features of depression. However, arguably, the most powerful empirical support for an etiological role for inflammation in depression comes from studies of patients with chronic Hepatitis-C infection treated with interferon-alpha (IFN-α)-based therapies, up to 50% of whom develop major depressive episodes [13]. Moreover, in patients with MDD, the presence of high levels of pro-inflammatory cytokines (in particular IL-6) [14] and acute phase proteins [15] suggest that inflammatory mediators might contribute to the pathophysiology of depression even in the absence of medical illness.

Far from being a unitary construct, depression is recognized to be a multicomponential disorder involving changes in motivation, cognition, attention, memory, and mood; as well as features such as disturbed appetite, sleep, and sexual dysfunction and physiological changes including cardiovascular and metabolic change. In studies of MDD, patients are typically recruited when symptoms are well established across each of these domains. However, following chronic IFN-α administration, individual features of clinical depression evolve with characteristic

time-courses allowing a unique opportunity to investigate the temporal evolution of individual symptom domains. For example, changes in mood, motivation, and fatigue (and in some cases feelings of social connection and spatial memory) can be readily observed within hours of IFN-α administration [16] and/or other experimental inflammatory challenges such as typhoid vaccination [11, 12, 17, 18] and LPS injection [8, 9, 19]. Changes in physiology, including altered central autonomic regulation of heart rate variability (similar to that observed in MDD) also occur acutely [20]. However in contrast, subjective reports of depressed mood, anxiety, and irritability assessed with clinical depression scales typically develop later, between the first and third months of IFN-α therapy [21].

This differential evolution of individual features of inflammation-associated clinical depression also provides a unique opportunity to characterize the neural circuitry underpinning specific components of inflammation-induced depression. To date, rodent and human brain imaging studies have successfully identified a discrete set of cortical and sub-cortical structures that appear particularly sensitive to changes in peripheral inflammation. These include the amygdala, striatum (particularly ventral regions), substantia nigra, insula, sub-genual and dorsal anterior cingulate, orbitofrontal cortex, and hippocampus/parahippocampus. Some of these structures appear to play relatively specific roles in particular aspects of inflammation-associated behavioral change. For example, actions on the ventral striatum [8, 22, 23] are associated with impaired reward sensitivity and hippocampus/parahippocampus acute memory impairment [4, 20] whereas other regions such as the insula, anterior and sub-genual cingulate, and amygdala appear to play broader less circumscribed roles [11, 16]. Common to many of these regions is that they form part of the extended limbic circuitry critical to complex motivational behavior, emotion, learning, and memory and the integration of behavioral and physiological allostatic responses to infection [24, 25].

Together, these studies are beginning to clarify how changes in peripheral inflammation are communicated to the brain. As discussed in more detail in the following sections they are also beginning to identify how actions of inflammation on discrete neural circuits induce individual components of this coordinated behavioral reorientation, which when chronic may evolve to MDD.

2 Communicating Inflammation to the Brain

In rodents, both the early central communication of peripheral inflammatory signals [26] and the subsequent motivational reorientation appear dependent upon the integrity of interoceptive visceral afferents traveling in the vagus nerve, visceral terminals of which express cytokine binding sites [27]. Early in the inflammatory response, antigen-presenting cells cluster in the vicinity of vagus nerve afferents and act as immune chemosensory elements signaling to vagal neurons via cytokine-dependent [28] and -independent mechanisms [29].

Immunohistochemical studies using the immediate early gene c-Fos to index neural activation confirm that peripheral inflammation and specifically binding of pro-inflammatory cytokines to vagus nerve receptors activate a network of brain structures implicated in homeostasis and the representation of internal bodily state (interoception) [26]. This afferent signaling is rapid; in the rat peripheral inflammation induces c-Fos expression in the primary projection nucleus of the vagus nerve (nucleus tractus solitarius – NTS) and secondary projection regions (including parabrachial, paraventricular and supraoptic hypothalamic nuclei, central amygdala, and bed nucleus of the stria terminalis) within an hour of peripheral inflammatory challenge [26].

In addition to signaling via the vagus nerve, central signaling of peripheral inflammation may also occur via interoceptive information conveyed via the spinal cord. For example, information traveling through spinal lamina I is predominantly tuned to motivationally salient sensations, including pain [30, 31], temperature [32], itch [33], and sensual touch [34], and converges with afferents traveling in the vagus nerve within the brainstem and thalamus [35]. In humans, cortical projections of convergent vagus and spinal interoceptive pathways to the posterior then mid/anterior insula cortex have been proposed to support a consciously accessible representation of physical wellbeing [36] and provide a neural substrate for subjective emotional feelings [30, 36].

We have previously demonstrated that this human interoceptive pathway is also activated by mild systemic inflammation [12]. Specifically, we demonstrated increased activity on fMRI within bilateral thalamic (basal (VMb) and posterior (VMpo) ventromedial nuclei) and dorsal mid and anterior insula components of this interoceptive pathway within 3 h of typhoid vaccine induced inflammation. The location of these activations is noteworthy as both the VMb (which receives predominantly vagal fibers) and VMpo (predominantly sympathetic inputs) project to dorsal mid/posterior insula in a rostrocaudal topographic manner (with vagal projections extending more rostrally) [30]. Following inflammation activations occurred in more rostral regions of interoceptive insula cortex than those reported for thermal sensation [32], noxious pain, and itch (non-vagal) but close to the region previously reported to be activated by antigen-induced airways inflammation in asthmatic patients [37].

Insula (and cingulate cortices) also played a key role in mediating subjective responses to inflammation, particularly fatigue (Fig. 1). For example, inflammation but not placebo-associated fatigue was predicted by activity changes within bilateral mid/posterior insula and right anterior cingulate (pACC/aMCC). These findings highlight a degree of specificity of the neural mechanisms underlying inflammation-associated fatigue that is not seen in more general placebo-associated fatigue (which might result from more heterogeneous mechanisms).

Previous studies showing insula responses to subjective experience of graded cooling [32], itch [38], and intensity of dynamic exercise [39] support the hypothesis that the subjective experience of inflammation-associated fatigue results from an insula-based interoceptive mechanism. This hypothesis has also been reinforced by three more recent studies. The first, using typhoid vaccine induced inflammation

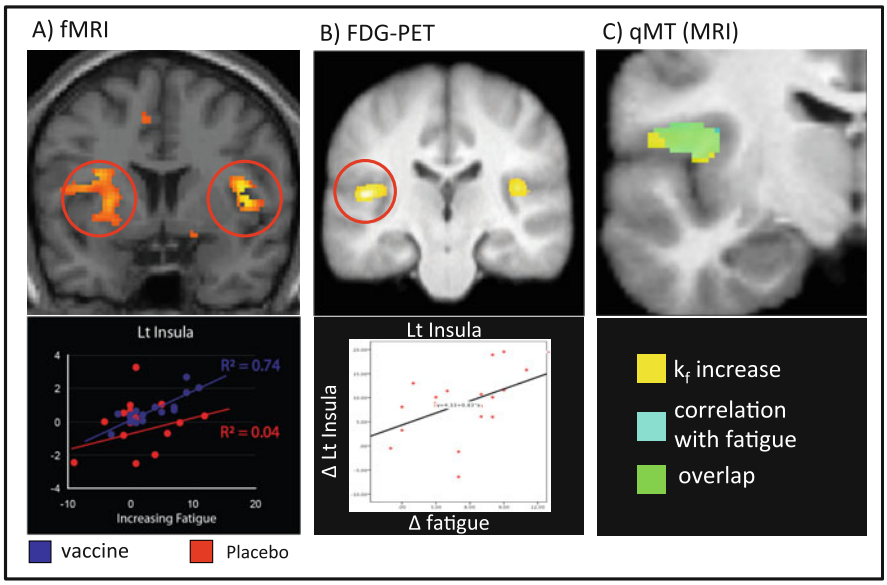

Fig. 1 Inflammation-induced insula activity predicts subjective fatigue. (**a**) Increase in bilateral insula activity on fMRI during performance of a color word Stroop task after inflammation compared to placebo. *Lower panel* shows that left insula cortex activity predicted experience of inflammation-induced fatigue (*blue*) but not fatigue associated with placebo (*red*). Data from Harrison et al. [12]. (**b**) Increase in bilateral resting glucose metabolism (FDG-PET) after typhoid vaccine induced inflammation compared to placebo. *Lower panel* shows correlation between change in left insula glucose uptake and subjective fatigue. Data from Harrison et al. [23]. (**c**) Left insula region showing an increase in magnetization transfer constant k_f after typhoid vaccine induced inflammation compared to placebo (*yellow*), correlation with fatigue (*blue*), and overlap of these regions (*green*). Data from Harrison et al. [23]

and fluorodeoxyglucose PET (FDG-PET) imaging, which replicated associations between changes in mid insula activity (in this case glucose metabolism) and the subjective experience of inflammation-induced fatigue [23]. The two other studies used a more potent model of inflammation (0.8–0.6 ng/kg LPS) and either FDG-PET or resting state fMRI which is a powerful technique for identifying functionally connected brain networks [40]. The first demonstrated an increase in right anterior insula glucose metabolism that correlated with loss of social interest (but not fatigue) [41] while the latter demonstrated correlations between subjective feelings of both inflammation-induced malaise and discomfort and heightened functional connectivity between the left anterior insula and mid-cingulate cortex [42]. Together, these studies highlight the importance of this interoceptive pathway projecting to insula in the central communication of inflammation induced using experimental models of bacterial infection. They also emphasize the likely importance of the insula in translating these interoceptive signals into negative subjective experiences of inflammation-induced fatigue and malaise (and possibly social disconnect) that develop early after the onset of inflammation.

Interestingly, patients with left or right insular strokes describe significantly greater subjective anergia with under activity and tiredness than patients with strokes sparing the insula region [43]. Furthermore, increased insula metabolism and altered interoceptive processing have also emerged as key features of MDD [44, 45]. Increases in insula (particularly anterior insula) glucose metabolism occur following sadness induction with a converse reduction in insula glucose metabolism observed following successful depression remission [44]. Insula functional connectivity [46] and regional homogeneity (ReHo) (a measure of the temporal homogeneity of neural activity within this region) are also impaired in MDD with the later correlating with retardation components of depression [47]. These findings have been interpreted as consonant with neurovegetative features of MDD (such as fatigue) and associated changes in autonomic function [44].

In addition to this neurally mediated pathway, LPS-induced peripheral inflammation also results in a rapid (within 3 h) and diffuse increase in the central nervous system (CNS) expression of translocator protein (TSPO) [48]. In the CNS, TSPO is predominantly expressed in activated microglial cells suggesting that systemic inflammation may be translated into a central microglial inflammatory signal through diffuse actions at the cerebrovascular endothelium. However to date, relatively few participants have undergone TSPO imaging post-LPS and no association has been identified between regionally specific increases in TSPO uptake and subjective experiences of inflammation-induced fatigue.

Curiously, activation of interoceptive projections to insula appears less critical in mediating subjective responses to inflammation induced using mimics of viral infection such as IFN-α. Indeed, IFN-α does not appear to be associated with substantial changes in insula microstructure or glucose metabolism either acutely [16] or when chronically administered [49] suggesting that visceral afferents may not be the principle pathway mediating IFN-α-induced fatigue. Why such marked differences exist between models of bacterial and virally induced infection is currently unclear though may be usefully informed by the pre-clinical rodent and non-human primate literature. For example, though IFN-α injection results in a rapid increase in circulating and cerebrospinal fluid (CSF) concentrations of type-I interferon [50] other pro-inflammatory cytokines such as IL-6, TNF-alpha, and IL-1 are only modestly elevated [16]. Furthermore, in rodents profound CNS induction of IFN-inducible genes is observed within hours of intraperitoneal IFN injection [51], indicating that IFN-α likely gains rapid access to the CNS where its actions may be more directly transduced.

To summarize, the dorsal insula represents the ultimate projection of interoceptive pathways and is believed to provide a cortical representation of all aspects of bodily physiology including changes in peripheral inflammation [24]. Progressive posterior to anterior projections are proposed to integrate and translate this information into experiential feeling states such as feelings of warmth, malaise, or fatigue. Experimental models of bacterially induced inflammation result in rapid structural and functional changes in posterior, mid, and anterior insula cortices that correlate with concomitant increases in subjective fatigue and malaise. Similar changes in insula function are also well described in MDD where they correlate

with neurovegetative symptoms of depression including fatigue illustrating striking commonalities between inflammation-induced fatigue/malaise and neurovegetative symptoms of depression.

3 Motivational Change

Impairment in reward-related behavior is a core feature of the motivational reorientation characteristic of both inflammation-induced sickness behavior [2] and idiopathic depression [52]. In the context of sickness, this motivational shift is proposed to efficiently prioritize whole organism responses to clearing the infecting agent. However when inflammation is severe or prolonged this persistent motivational reorientation may predispose to the development of MDD [1].

A wealth of human and rodent studies have identified the ventral striatum as a critical structure for mammalian reward-related processing and appetitive motivation [53]. Single cell recordings demonstrate that a subgroup of dopaminergic cells within the midbrain encode a reward prediction error, increasing (or decreasing) firing rate if a reward is higher (or lower) than predicted [54]. Dopaminergic projections from the midbrain to the ventral striatum serve to update estimates of the value of different available options and bias behavioral choice so that long-term future reward is maximized. Reinforcement learning algorithms such as temporal difference models [55] have provided a powerful framework for modeling this dopaminergic prediction error signal that is proposed to mediate learning of associations between stimuli, responses, and outcomes [56]. They also allow interrogation of brain imaging data to identify brain regions whose activity correlates with these reward prediction error signals [57]. Dopamine firing may also contribute to "incentive salience," the process by which a stimulus grasps attention and motivates goal-directed behavior by its association with reinforcing events [58].

Interestingly, both MDD patients [59] and previously healthy participants given an inflammatory challenge show a reduction in ventral striatal responses to reward outcomes [22, 60]. Inflammation has also been linked to acute reductions in ventral striatal reactivity to cues predicting rewards [19], though this is less convincingly reported in MDD [60]. In the context of inflammation, reduced ventral striatal responses to both reward cues and reward outcomes also correlate with induced anhedonia [19, 22]. Patients with MDD have also been shown to exhibit reduced reward prediction error encoding in the striatum and midbrain [59]. This change also correlated with the severity of anhedonia symptoms suggesting that abnormal encoding of prediction errors in MDD could result in anhedonia by altering the learning and salience of rewarding events [59].

Using a similar probabilistic instrumental learning task we have recently demonstrated that mild inflammatory challenge (induced with typhoid vaccination) also results in a relative impairment in sensitivity to rewards compared to punishments [61]. Furthermore, this motivational reorientation was associated with opposing actions on ventral striatal reward (and right anterior insula punishment) prediction

error encoding [61]. Similar to patients with MDD, inflammation was associated with reduced striatal reward prediction error encoding. Behaviorally, after inflammation participants in this study also showed a reduced propensity to choose rewarded options but enhanced avoidance of punished ones. This behavioral change was captured computationally as a significant condition (gain, loss) by inflammation (vaccine, placebo) interaction for the subjective value of rewards compared with punishments.

Though dopamine activity was not measured in this (or Eisenberger's study demonstrating effects of inflammation on reward cues), a similar reduction in striatal reward prediction error magnitude (and propensity to choose the most rewarded action) has been reported on this task after haloperidol (a dopamine receptor-2 antagonist) [57]. This suggests that effects of inflammation on striatal prediction errors were likely mediated by actions on dopamine release. Supporting this, inflammation has been linked to altered nucleus accumbens dopamine efflux in rodents [62] and disrupted presynaptic dopamine synthesis/release in humans [22]. After LPS challenge monkeys also exhibit significantly lower cerebrospinal fluid concentrations of the dopamine metabolite homovanillic acid [50].

However, exactly how inflammation modulates dopamine function is currently unclear. Cytokines such as interferon-alpha have been shown to inhibit dopamine synthesis by reducing CNS tetrahydrobiopterin, an essential cofactor for tyrosine hydroxylase, the rate-limiting step in dopamine synthesis [63]. Inflammation can also decrease synaptic dopamine by increasing expression of the monoamine reuptake transporter [63–66]. Inflammation may further influence dopamine neurotransmission via activation of the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase and resultant formation of neurotoxic kynurenine metabolites [1].

To date most studies investigating the basis of the motivational reorientation associated with inflammation have focused on reward-related processing. However, in our recent study we also showed that inflammation significantly enhances sensitivity to punishment. This was captured in the data modeling as a significant increase in the subjective (negative) value of punishment; i.e., the magnitude of the potential punishment was experienced as being greater after inflammation [61]. This behavioral change was also associated with greater encoding of negative punishment prediction error in the right anterior insula.

Increasing punishment prediction error is one way to increase the subjective value of punishment and may serve as the computational mechanism by which the anterior insula drives this improvement in avoidance behavior. This interpretation is in keeping with theories proposing that brain areas like the insula involved with somatic affective representations (as discussed above) are causally involved in choice behaviors [24, 30, 57]; particularly in the context of potential losses [67]. This association between punishment sensitivity and insula activity also complements an earlier study showing impaired punishment sensitivity in patients with selective insula lesions [68] and suggests that relative sensitivity to reward versus punishment is a state rather than a trait-dependent attitude; flexibly enhancing loss minimization in the context of a serious threat (such as an infection) yet maximizing responses to gains when in good health.

As predicted by models of learned helplessness, dysfunctional responses to negative feedback were some of the earliest cognitive changes described in depression [69]. More recently, meta-analysis of computationally modeled reinforcement learning tasks in patients with current or past history of MDD has reported a selective reduction in subjective reward value (rather than reward learning rate) [52] similar to what has been observed in the context of inflammation. Relatively selective actions on reward/punishment magnitude have also been reported following dopamine manipulation and insular damage [57, 68]. This rapid cognitive adaptation following inflammation serves to heighten relative sensitivity to punishment versus reward raising the intriguing possibility that while this may be beneficial in the context of an infective challenge when metabolic resources are diverted to fighting the infecting organism, when chronic, it may predispose to developing the maladaptive changes in motivation observed in depression. Evidence for common neural mechanisms mediating motivational change and anhedonia in MDD and inflammation have been further strengthened by a recent paper in unmedicated MDD patients showing that decreased connectivity between the ventral striatum and ventromedial prefrontal cortex (vmPFC) also mediates observed associations between raised CRP and anhedonia [70].

4 Psychomotor Retardation

Psychomotor retardation, defined as a slowing-down of thought and physical movements, is a core feature of depression [71] and readily induced following systemic administration of pro-inflammatory cytokines or other experimental models of inflammation [72].

In rodents, systemic administration of bacterial LPS or inflammatory cytokines (notably IL-1β) consistently suppresses locomotor and motivational behaviors, resulting in increased periods of immobility [73]. These depressant effects of peripheral IL-1β on behavior are potentiated by IL-6 [74] and motor-suppressing effects of inflammatory challenge are reduced by antibodies against IL-6 [75, 76] and attenuated in IL-6 knockout mice [77]. Psychomotor retardation expressed as prolonged motor reaction times is also a feature of human sickness behaviors [78, 79], and is observed even after relatively mild inflammatory challenges [10].

In the first study to directly investigate the neural mechanisms underlying the psychomotor consequences of peripheral inflammation Brydon et al. [10] recorded whole brain responses to performance of a simple motor task (button press). They demonstrated that low-level inflammation (induced using typhoid vaccine) selectively modulated substantia nigra reactivity to performance of a button press task during both a low-level visual stimulation (flashing checkerboard) task and a more demanding cognitive (color word Stroop) task. They also observed a striking correlation between peripheral IL-6 responses and motor response time on the Stroop task for both low level (congruent) and attentionally demanding (incongruent) trials suggesting an action on low-level pre-cognitive processes. Supporting

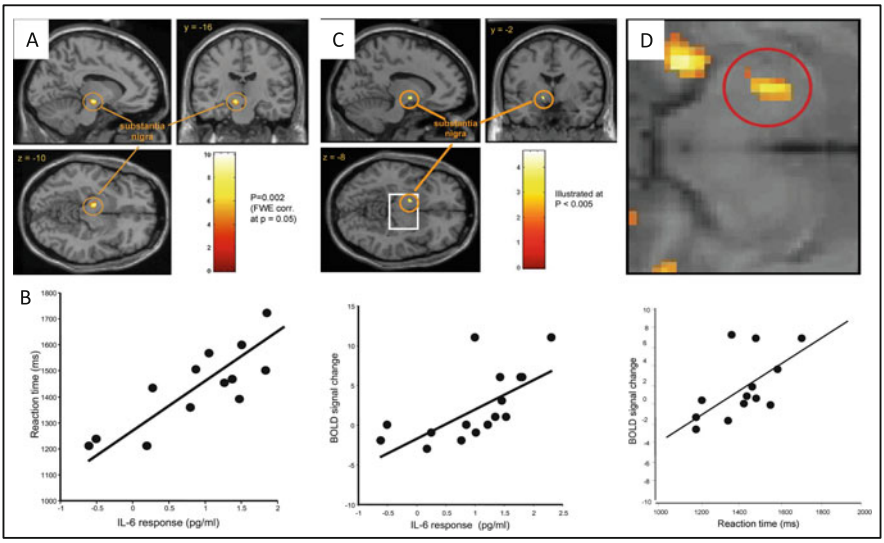

Fig. 2 Inflammation induces psychomotor slowing through actions on substantia nigra. (**a**) Effect of inflammation on substantia nigra reactivity during a low-grade visual stimulation task (flashing checkerboard). (**b**) Correlation between IL-6 response to typhoid vaccination and reaction time on a color word Stroop task. (**c**) Correlation between IL-6 response to typhoid vaccine and increase in left substantia nigra activity during Stroop task performance. (**d**) Correlation between mean Stroop reaction time and left substantia nigra reactivity following inflammation. Reproduced from Brydon et al. [10] with permission

this, both peripheral IL-6 responses and changes in left substantia nigra reactivity predicted inter-individual differences in sensitivity to the motor impairing effects of inflammation (Fig. 2).

Located in the midbrain, the substantia nigra is the major source of dopamine in the brain with striatal projections playing a pivotal role in the facilitation of movement [80]. Nigral dopaminergic projections within striatal target regions also modulate sensorimotor processing in response to stimulus salience [81] and have been linked to the reduction in novelty salience observed during inflammation [17]. Agonists that potentiate dopaminergic neurotransmission improve the speed of motor responses in animals, whereas striatal dopamine depletion and selective blockade of dopamine D1 or D2 receptors have been shown to significantly impair performance on reaction time tasks [82, 83]. Similar to effects of inflammation, impaired task performance following inhibition of dopamine is due to lengthened response latencies rather than deficits in the accuracy of responses [83]. Lower levels of striatal dopamine transporter have also been associated with slower motor reactions in healthy elderly humans [84]. Brydon's results extend these observations and provide empirical evidence for involvement of the dopamine system in behavioral consequences of peripheral inflammation, highlighting a role for IL-6 and substantia nigra neural activity in infection-related psychomotor impairments.

As discussed in the previous section, brain dopamine levels are modulated by peripheral administration of IFN-α and other inflammatory cytokines in rodents [66]. Human patients receiving IFN-α-based immunotherapy also experience marked psychomotor slowing [72] which has been shown to correlate with abnormalities in left dorsal striatal glucose metabolism [49]. These patients also demonstrate bilateral reductions in striatal 18F-DOPA turnover on PET imaging suggesting decreased presynaptic dopamine synthesis or release, though associations with psychomotor responses were not reported in this study [22]. Interestingly, elevated circulating IL-6 and altered striatal dopaminergic neurotransmission are also associated with psychomotor slowing in people with MDD [85, 86]. However, these studies have tended to focus on changes within striatal projection regions (rather than the substantia nigra) that are typically easier to image in human functional imaging studies. Interestingly, a recent study has shown that older individuals appear particularly susceptible to the psychomotor effects of IFN-α treatment. In this study, increased choice movement time correlated with changes in both peripherally induced TNF and left basal ganglia glutamate (reflected by glutamate/creatine ratio (Glu/Cr)), the other major neurochemical input to the striatum [87].

Correspondingly, decreased left striatal presynaptic dopamine function has been described in depressed patients presenting with marked psychomotor retardation [86]. Left dorsal striatum (caudate) lesions are also associated with a higher frequency and severity of post-stroke depression [88]. In their study using 18F-DOPA PET Martinot et al. [86] demonstrated a reduction in left caudate tracer uptake in MDD patients with psychomotor retardation but not MDD patients with high impulsivity or comparison control participants, providing direct evidence of a link between striatal dopamine hypofunction and psychomotor retardation. The importance of the left dorsal striatum to psychomotor retardation associated with MDD and inflammation has been further strengthened by a recent paper demonstrating a link between plasma and CSF levels of C-reactive protein (CRP), left basal ganglia glutamate, and psychomotor slowing in untreated depressed patients [89].

Together, these data support a central role for bottom-up dopaminergic (substantia nigra) and top-down glutaminergic inputs into the dorsal striatum in both inflammation- and MDD-associated psychomotor retardation. Curiously, most of these studies also report strikingly left lateralized effects. Though robustly reported this finding is currently poorly understood and will require further investigation in future studies. During an infection, psychomotor slowing may serve to minimize energy expenditure and conserve heat, thereby enhancing immune function. Convergent findings in inflammation and MDD-associated psychomotor retardation suggest that chronic activation of these mechanisms may differentiate MDD patients presenting with predominant psychomotor retardation or impulsivity/anxiety symptoms. Whether this difference in presentation also relates to differences in peripheral inflammatory markers is the focus of ongoing studies.

5 Autonomic Responses

Physiological changes including hyperactivity of the hypothalamic-pituitary-adrenal axis [90] and disturbance in the autonomic control of the heart and vasculature [91] are another feature of MDD, with the later proposed to mediate the relationship between MDD and risk for cardiovascular disease. Inflammation is also increasingly implicated in cardiovascular disease and has been highlighted as a potentially modifiable risk factor by the American Heart Association (AHA) and Centers for Disease Control (CDC) [92]. In this context it is therefore noteworthy that, in addition to impairing mood, experimentally induced inflammation can also perturb both local cardiovascular reactivity [93] and the central autonomic control of the cardiovasculature [20].

One of the most consistent physiological changes reported in MDD is a change in heart rate variability (HRV) which provides an index of beat-to-beat changes in heart rate [91]. Briefly, high frequency (HF) variation in heart rate is mediated by parasympathetic tone and is believed to maintain cardiac stability and protect against myocardial infarction and heart failure. In contrast low frequency (LF) variation tends to reflect sympathetic tone which is associated with an increased risk of malignant arrhythmias and sudden cardiac death. The ratio of these measures (LF/HF) provides a composite measure of HRV with lower values reflecting healthy cardiac function [94]. Meta-analysis demonstrates a significantly higher LF/HF ratio in MDD patients, suggesting an increase in sympathetic and reciprocal decrease in parasympathetic activity [91] that may underlie the association with increased cardiovascular risk.

A similar acute increase in LF/HF ratio has also been observed following induction of mild inflammation using typhoid vaccination [20] and shown to mediate associated changes in blood pressure. Within the brain, inflammation-induced shifts in LF/HF balance are associated with changes in glucose metabolism (FDG-PET) within three discrete regions: dorsal anterior cingulate, posterior cingulate, and pons. Each of these regions is implicated in regulating stressor-evoked blood pressure reactivity. Their recruitment following inflammation and role in mediating effects on blood pressure further illustrates how central effects of inflammation can also contribute to peripheral physiological changes. Together, these findings demonstrate that commonalities between MDD and inflammation extend beyond mood and motivational changes to include changes in physiological function and highlight the brain mechanisms that bind psychological and physiological wellbeing in these conditions.

6 Attention and Executive Function

Cognitive alterations are a prominent feature of sickness behaviors and manifest predominantly as disturbances in attention, memory, and higher-level executive function [4, 95]. Following experimentally induced influenza infection performance is impaired on oddball type tasks that demand sustained attention, though hand-eye coordination and logical reasoning are unaffected [95]. Similar impairments on attentionally demanding tasks are also observed after acute [96] and chronic administration of interferon-alpha [78]. Patients receiving chronic interferon-alpha also show impaired performance on tests of cognitive speed, verbal memory, and executive functions indicating likely impairment of frontal-sub-cortical brain function [97]. This interpretation is supported by EEG which shows focal changes in frontal lobe regions after acute IFN-alpha administration [98].

Cognitive impairments are an important feature of MDD with deficits particularly prominent in measures of sustained attention, memory, and executive function [99, 100]. Deficits in executive function appear wide-ranging and include impairments in inhibition, set shifting, updating, working memory, and planning indicating frontal-sub-cortical dysfunction [100]. Focusing on inhibition, which has been most closely investigated in inflammation, meta-analysis of color word Stroop studies (where target words are presented in congruent or incongruent font colors) shows a relatively selective impairment on incongruent trials that demand inhibition of pre-potent lexical responses [100]. Studies investigating this effect using neuroimaging suggest that MDD patients show greater interference because they fail to adequately recruit left dorsolateral prefrontal cortex (DLPFC) [101] or require greater left DLPFC activation to achieve performance levels observed in controls [102]. Interestingly, mid-DLPFC is implicated in selecting and biasing attention to the most task-relevant representation indicating a potential circumscribed cognitive deficit in MDD patients [100].

In the first neuroimaging study of inflammation and cognition Capuron et al. [103] used a variation of the CANTAB response time (RT) task to investigate effects of IFN-α on visuospatial attention. Interferon-alpha did not impair task performance or recruitment of the parieto–occipital attention network. However, IFN-α treated patients did recruit an additional dorsal anterior cingulate cortex (ACC) region that was not observed in controls. ACC is implicated in conflict monitoring and its activation is proposed to reflect the degree of intentional effort or willed control needed to perform a task [104]. ACC activation in IFN-treated patients therefore potentially reflects a need to exert greater cognitive control to maintain normal levels of task performance in the face of inflammation.

Interestingly, a similar pattern of behavioral and neuroimaging changes also emerged in a study using the color word Stroop task to investigate effects on mild inflammation (typhoid vaccination) on cognitive inhibition [12]. Again, inflammation was not associated with any significant change in task performance. However, it did have a striking impact on the network of brain regions recruited during

attentionally demanding incongruent trials (that require inhibition of pre-potent responses) including DLPFC and mid-cingulate (aMCC/pMCC) cortices. Because participants showed no performance differences, these effects likely represent a need for additional neural resources to maintain task performance under inflammation. As mentioned above, activity within both DLPFC and dorsal ACC is typically enhanced with increasing cognitive demands. Both regions also show an increase in activity during performance of a cognitively demanding visual task in the face of cross-modal auditory distracters [105]. Their concurrent activation in states of inflammation therefore suggests that interdependent cognitive/attentional [106] and somatic (autonomic) [107] mechanisms may be invoked to maintain performance in the face of increased conflict from interoceptive processing.

In sum, these studies indicate the need to recruit additional neural resources, notably DLPFC and ACC to maintain cognitive performance in the face of systemic inflammation. Similar to findings observed in MDD, this is particularly marked in tasks with high attentional demand or requiring inhibition of pre-potent responses. Exactly how closely the cognitive deficits induced by inflammation relate to those observed in MDD is yet to be fully determined. Future research will need to further clarify the origin of impairments in executive function observed in MDD and dissect the neurobiological and cognitive mechanisms underlying the broad cognitive deficits reported in both MDD and inflammation. Neurobiological differences including changes in inflammatory state and its impact on neurotransmitters such as serotonin or kynurenine breakdown products are one possible cause for these changes and recent studies are now beginning to characterize this [108].

7 Memory

Work in rodents has demonstrated that inflammatory cytokines modulate a number of neuronal processes including long-term potentiation (LTP) [109, 110], synaptic plasticity [111], and neurogenesis [112] that are critical to learning and memory. In health, immune mechanisms play a role in each of these processes and contribute to the remodeling of neural circuits that promote learning and memory [4, 111]. However, during systemic infection this positive regulatory function is disrupted resulting in acute memory impairments [113]. When inflammation is severe, cognitive impairment may also become persistent [114] and when it is chronic typical age-related impairments in cognition are accelerated [115].

Medial temporal lobe (MTL) structures appear to be particularly sensitive to the effects of inflammation. This may reflect their relatively high receptor and messenger RNA expression for pro-inflammatory cytokines [116, 117] and their connectivity to regions such as the insula [118] that support cortical representations of peripheral inflammatory states [24]. Rodent studies have particularly emphasized the role of the hippocampus in inflammation-associated memory impairments. For example, IL-1 administration into the hippocampus selectively impairs spatial and contextual memory processes and contextual, but not (hippocampus independent)

auditory-cued, fear conditioning [4, 119, 120]. Similarly, over-expression of IL-1 mRNA within the hippocampus has been associated with delayed acquisition of spatial memory [120]. LTP is arguably the key neuronal mechanism for synaptic plasticity that underlies memory encoding and recall. It is therefore noteworthy that IL-1 compromises both hippocampal and dentate gyrus LTP [109, 121, 122]. Peripheral inflammatory challenges also induce IL-1 expression within brain regions, including the MTL [123] and can replicate many of the direct actions of inflammatory cytokines on MTL-dependent memory [124, 125].

In humans, individuals suffering from acute flu-like symptoms have been shown to exhibit impaired memory on tests of immediate and delayed verbal (and delayed picture) recall [126]. However, retrieval of semantic information consolidated in long-term store is unimpaired [126]. Similarly, inflammation induced experimentally with LPS impairs both verbal and nonverbal, declarative memory [9]. Importantly, these memory impairments remain prominent even when mood normalizes suggesting an effect induced by inflammation rather than being secondary to associated changes in mood [9]. Impaired verbal memory is also reported following IFN-α [97]. These studies, which have focused on declarative memory, broadly support the MTL sensitivity to inflammation observed in rodents. However, marked differences in the memory testing paradigms used in rodents and humans limit finer-grained translational inferences.

This difficulty has been partially mitigated (in the context of spatial memory) by the recent translation of the Morris water maze, used extensively to assess rodent spatial memory, for human use [127]. This task requires participants to remember the identity and spatial location of objects in a virtual arena and has been recently used to demonstrate that low-level inflammation also selectively impairs human spatial memory [18]. In particular, inflammation was associated with a selective impairment in remembering object location but not object identity. Furthermore, inflammation did not impair motor skill learning (indexed by performance on a mirror tracing task), a form of procedural memory that relies on a separate dorsal striatum-based memory system independent of the MTL [127]. Interestingly, inflammation was also associated with bilateral reductions in MTL resting glucose metabolism (measured using FDG-PET) with changes in the right parahippocampus significantly mediating the inflammation-induced impairment in spatial memory [18] (Fig. 3). The location of this effect is noteworthy as studies in rodents associate similar spatial memory impairments with changes localized to the hippocampus not the parahippocampus [4].

Why these inter-species differences exist may be usefully informed by human lesion studies, that show that human performance on Morris water maze type tasks and direct tests of object-location memory can be more strongly dependent on right parahippocampal than hippocampal integrity [128, 129]. Right parahippocampal activity during object-location encoding has also been shown to predict subsequent retrieval success with a spatial cue [130]. Furthermore one-trial memory for object-place associations also appears to be critically dependent on posterior parahippocampus rather than the hippocampus in monkeys [131].

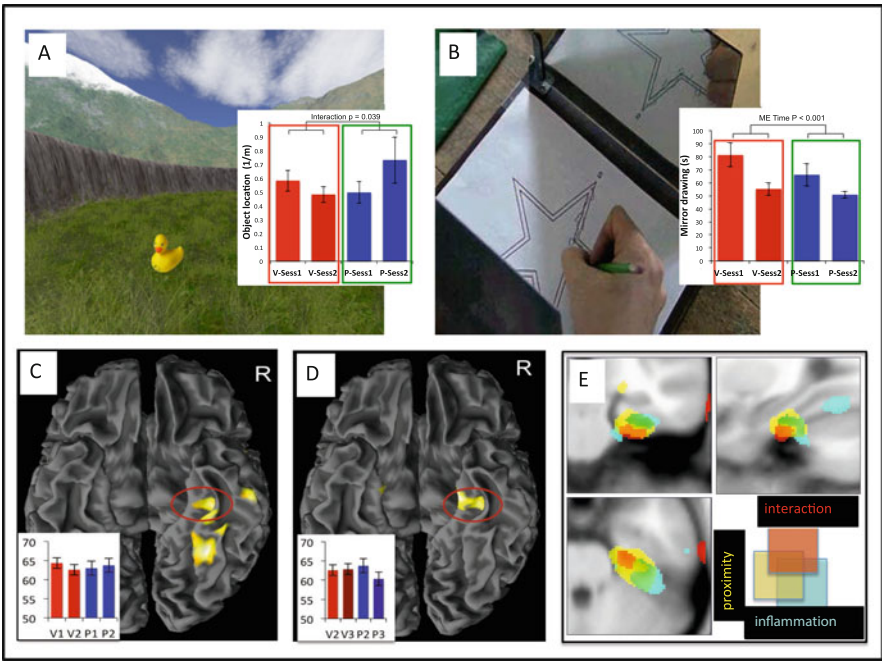

Fig. 3 Inflammation impairs spatial memory via actions on medial temporal lobe glucose metabolism. (**a**) Virtual reality object-location task. *Inset* shows that object-location accuracy (*y* axis) improves after placebo (*red*) but deteriorates after typhoid vaccine induced inflammation (*blue*). (**b**) Control mirror tracing task. *Inset* shows equivalent improvement in performance after both placebo (*red*) and vaccine (*blue*). (**c**) Decrease in medial temporal lobe (MTL) fluorodeoxyglucose (FDG) uptake in participants given typhoid vaccine after scan 1 (V1 to V2 *red*) compared to controls (*blue*). (**d**) Similar reduction in MTL FDG uptake in participants given typhoid vaccine after scan 2 (P2 to P3 *blue*). Note participants given typhoid vaccine after scan 1 (*red*) show a sustained reduction in FDG uptake at scans 2 and 3 (V2 to V3). (**e**) Reduction in MTL FDG uptake after typhoid vaccination (*blue*), correlation between object-location accuracy and FDG uptake (*yellow*), and area correlating with interaction between task performance and inflammation illustrated in **a** (*red*). Data from Harrison et al. [18]

Episodic memory is also one of the cognitive functions most susceptible to depression suggesting a relatively selective impairment in MTL function [132, 133]. Supporting this, meta-analyses of structural MRI studies have shown an 8–10% reduction in hippocampal volume in MDD [134, 135]. Similar to participants receiving LPS or experiencing flu-like symptoms, studies conducted on large populations of MDD patients (>8,000) report impaired performance on the delayed paragraph recall test of verbal declarative memory [136]. MDD patients have also been shown to perform significantly worse on a virtual reality measure of spatial memory [137]. Though to date most studies investigating memory function in MDD have focused on the hippocampus, evidence suggests that MDD may also be associated with abnormalities in broader MTL structures [138]. Future studies characterizing the specific pattern of memory deficits associated with MDD and

inflammation and their underlying neural substrates will be essential to furthering our understanding of how inflammatory processes contribute to the memory deficits observed in MDD.

8 Social Responses

Another feature of sickness behaviors is social withdrawal and social disconnection [139]. Feelings of social disconnection (experienced as loneliness) contribute to the development and maintenance of depression [140]. The observation that inflammation-induced social withdrawal can be reversed by antidepressant treatment [141] has motivated a number of recent human studies seeking to understanding how inflammation leads to social disconnection as a way of potentially understanding the mechanistic relationship between inflammation and depression.

In an early study, Eisenberger et al. [8] showed that inflammation increases feelings of social disconnection and furthermore that this change mediated the relationship between inflammatory activity and depressed mood. The questionnaire used to assess feelings of social disconnection in this study included items reflecting both a desire to withdraw socially and items that reflected a feeling of being socially isolated or disconnected from others. Both types of items were altered following LPS suggesting potentially dissociable effects on motivational processes ("I want to be alone") as well as processes involved in social cognition and social perception. Addressing this, Moieni et al. have recently demonstrated that LPS induced inflammation impairs performance on the "Reading the Mind in the Eyes" test of theory of mind [142]. This task evaluates how accurately participants can identify another's emotional state by looking only at their eyes [143] and suggests that in addition to effects on motivation, inflammation can additionally alter social processes central to our ability to correctly infer others mental and emotional states.

Another important feature of MDD is that it is twice as common in women as men. One factor proposed to mediate womens' increased vulnerability is their greater exposure and reactivity to interpersonal stressors [144]. It is therefore noteworthy that though women do not show convincingly greater pro-inflammatory cytokine responses to LPS they do report greater increases in feelings of social disconnection and depressed mood suggesting that inflammation may play a role in mediating sex differences in rates of MDD [145]. This is supported by another study that combined LPS and the Cyberball task during fMRI to investigate effects of inflammation on social exclusion [146]. In this study, LPS-induced increases in IL-6 correlated with increased activity within a matrix of brain regions including dorsomedial prefrontal cortex (PFC), posterior superior temporal sulcus (STS), dorsal anterior cingulate (dACC), and insula that are implicated in social processing. However, though this relationship was observed across all participants only in women did it significantly mediate associations between inflammation and depressed mood.

Brain regions such as the posterior superior temporal sulcus (pSTS) and medial prefrontal cortex are strongly implicated in tasks of social cognition that involve extracting socially meaningful information and inferring another's mental state [147]. It is therefore noteworthy that each of these regions showed heightened activity in proportion to induced inflammation [146]. This interpretation is also in keeping with reported impairments in performance on the "Mind in the Eyes" test on theory of mind [142]. Supporting these findings, inflammation has also been shown to disrupt the functional connectivity of both the pSTS and medial prefrontal cortex to sub-genual cingulate (sACC) (a region central to integrating social, emotional, and physiological responses) during an implicit emotional face processing task [11]. Together, these studies demonstrate effects of inflammation on social processing including increased feelings of social disconnection and impaired theory of mind. Accumulating evidence suggests that in some participants, particularly women, these changes may mediate associated impairments in mood and illustrate a potential mechanism linking inflammation to depression.

9 Network Connectivity

The preceding sections have focused on dissecting effects of inflammation and MDD on discrete emotional, cognitive, behavioral, and physiological changes and relating this to regional changes in brain function. However, a small number of recent studies have begun to look at the effects of inflammation on network connectivity, driven by the recognition that even the simplest cognitive functions involve highly distributed processing [148]. The first such study used a simple psychophysiological interaction (PPI)-based approach to investigate the effects of systemic inflammation on connectivity of the sub-genual cingulate (sACC) [11]. In so doing, this study showed that inflammation-associated changes in total mood modulated not just sACC activity but also its functional connectivity to the nucleus accumbens, amygdala and superior temporal sulcus, regions central to the processing of reward, and emotionally and socially salient information, respectively. Furthermore, inflammation-induced reductions in the effective connectivity of the sACC to each of these regions predicted the associated deterioration in total mood.

This study is noteworthy as the sACC is recognized as a key node in functional and anatomical models of mood regulation [149] and the coordination of emotional processing. It is also strongly implicated in the pathophysiology of MDD [44]. Increased sACC activity seen in depression has also been shown to reverse with successful depression treatment with a selective serotonin reuptake inhibitor [44, 150], deep brain stimulation [151] of adjacent white matter tracts, and even placebo [150]. Importantly, the sACC is a region that is strongly implicated in integrating multiple components of mood homeostasis. Its recruitment in inflammation-induced mood change suggests that inflammation-associated changes

in mood recruits a network of brain regions similar to that implicated in primary depression.

More recently three further studies have investigated the effects of inflammation on brain functional connectivity networks observed at rest. In the first using low dose LPS (0.4 ng/kg), seed-based analysis revealed a rapid and widespread reduction in the functional coupling of the amygdala, insula, and cingulate cortices to multiple brain networks involved in affective-emotional, motivational, and cognitive-modulatory processes [152]. Similar to the PPI analysis of task-related fMRI [11], LPS was associated with reduced connectivity between the amygdala and prefrontal structures, though these authors were unable to show any significant relationship to changes in mood. The second study used a slightly higher dose (0.6 ng/kg) of LPS and specifically investigated effects on connectivity between anterior and posterior insula seeds and orbitofrontal and cingulate (anterior and middle). This study identified a specific increase in left anterior insula to left mid-cingulate cortex that additionally predicted LPS associated back pain and global sickness [42]. These regions form key components of the pain matrix, and it is noteworthy that they have also been previously linked to LPS induced increases in visceral pain sensitivity [153].

The third study adopted a different approach and investigated effects of IFN-α on measures of network function derived from graph theory [154]. Briefly, graph theory provides a powerful mathematical approach for analyzing the structure of complex networks, and application to the human brain has revealed insights unavailable from conventional approaches. For example, it has shown that similar to other complex networks, the brain utilizes an efficient "small-world" connectivity architecture that serves to minimize wiring cost while maintaining robustness to random damage to individual regions (nodes) or connections (edges) [155]. Within 4 h of administration, IFN-α was associated with a striking reduction in global network connectivity and network efficiency indicating a global reduction in information transfer among nodes forming the whole brain network. Furthermore, these changes in global network connectivity and efficiency of information exchange correlated strongly with IFN-α induced changes in mood, confusion, fatigue, and tension/anxiety.

How peripherally administered IFN-α or LPS can so rapidly impair the functional connectivity of such large-scale brain networks is currently uncertain. However, the observation that these actions are effected on a global scale points towards a likely role for neuromodulators such as dopamine, norepinephrine, or serotonin that can rapidly alter diverse and widespread neuronal populations rather than a more regionally targeted effect. In support of this, inflammation has been linked to altered nucleus accumbens dopamine efflux in rodents [62], decreased striatal dopamine release in rhesus monkeys [156, 157], and reduced presynaptic dopamine synthesis or release in humans [22]. Further, monkeys showing behavioral impairment after inflammatory challenge with lipopolysaccharide exhibit significantly lower cerebrospinal fluid concentrations of the dopamine metabolite homovanillic acid [50].

Network-based analyses have also been pursued in MDD and, similar to the findings of post-LPS, have shown reduced functional connectivity within broad prefrontal-limbic-thalamic areas, particular regions sub-served by the left amygdala-ACC and the right insula–precuneus Labrenz et al. [158]. Graph theoretic analyses of MDD have also demonstrated changes in network topology including abnormal small-world organization and network efficiency [159]. Though application of advanced connectivity analyses to both MDD and inflammation remains in its relatively infancy, similar marked changes in network connectivity, particularly within prefrontal and limbic regions across conditions, support the utility of this approach as a way of characterizing the relationship between inflammation and MDD. Demonstration of often-marked correlations between global measures of network function and mood/cognition suggests a likely role for neuromodulators such as dopamine and serotonin, that are implicated in the pathophysiology of both MDD and sickness behaviors. Further characterization of these associations will require combination of network-based analyses with PET imaging of specific neuromodulators and/or metabolomic approaches.

10 Summary

Rodent and human brain imaging studies have been successful in characterizing a discrete set of cortical and sub-cortical structures sensitive to changes in peripheral inflammation and highlighted their role in many of the key components of sickness behavior. These regions show a striking similarity to the network of areas implicated in the mood, motivational, and cognitive deficits characteristic of MDD. In the context of models of bacterial infection, peripheral inflammation rapidly recruits an interoceptive pathway projecting to insula. This pathway and its terminal projection to the insula provides a central representation of all aspects of bodily physiological state and its translation into consciously accessible feeling states. Following inflammation, recruitment of the insula cortex is implicated in feelings of fatigue and malaise (and possibly social disconnection) as well as a heightened sensitivity to punishment. Bidirectional connections between the insula and the anterior and mid-cingulate cortex provide a substrate for the heightened sensitivity to visceral and pressure pain. Similar to MDD, actions of inflammation on ventral striatal reward processing and reward prediction error encoding appear to underlie shifts in reward sensitivity and motivation. Cognitive deficits (particularly impaired MTL dependent memory and performance on attentionally demanding tasks) are characteristic of both MDD and inflammation and relate to disrupted hippocampal/parahippocampal processing and DLPFC, respectively. More recent functional connectivity studies highlight the distributed nature of cognitive processes and hint at the likely importance of broadly acting neuromodulators like dopamine and serotonin in the mood, motivational, and cognitive impairments characteristic of both MDD and inflammation.

Based on these foundations, future studies combining multiple functional neuroimaging techniques with metabolomic and proteomic approaches should help us move closer to the goal of a mechanistic understanding of the relationships between peripheral inflammation, regional brain structure/function, and discrete cognitive phenotypes observed in MDD.

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Role of Kynurenine Metabolism Pathway Activation in Major Depressive Disorders

Jonathan Savitz

Abstract A proportion of depressed individuals show evidence of inflammation. Both animal, quasi-experimental, and longitudinal studies indicate that inflammatory processes may play a causal role in the developmental of depressive illness. While there may be multiple causal pathways through which inflammatory processes affect mood, activation of the kynurenine pathway is essential for the development of depression-like behavior in rodents. Studies of hepatitis C or cancer patients receiving treatment with inflammation-inducing medications show increased activation of the kynurenine pathway and decreased levels of tryptophan that correlate with inflammation-induced depression. Further, this treatment has been shown to lead to increased production of neurotoxic kynurenine pathway metabolites such as quinolinic acid (QA). Similarly, in non-medically ill patients with major depression, multiple studies have found activation of the kynurenine pathway and/or preferential activation of the neurotoxic (QA) pathway at the expense of the production of the NMDA antagonist, kynurenic acid. Initially, activation of the kynurenine pathway was believed to precipitate depressive symptoms by depleting brain serotonin, however, the weight of the evidence now suggests that an imbalance between neurotoxic and neuroprotective metabolites may be the principal driver of depression; conceivably via its effects on glutamatergic neurotransmission.

Keywords Depression • Glutamate • Hippocampus • Inflammation • Kynurenic acid • Kynurenine • Prefrontal cortex • Quinolinic acid • Tryptophan

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Contents

1 Introduction

Major depression has a lifetime prevalence of ~17% in the USA [1], is the second largest source of years lived with disability for Americans [2], significantly increases the risk of suicide [3], decreases life expectancy among people in the general adult population [4], and has been estimated to cost the economy $200 billion [5]. Yet knowledge of this disorder's pathogenesis remains limited and the efficacy of existing treatments is modest. When a stringent criterion for treatment efficacy is used, namely that patients achieve "remission," (defined as having a depression rating scale score in the non-depressed range by study end), 1/3 of patients achieve remission on a selective serotonin reuptake inhibitor (SSRI) compared to 1/4 on placebo giving a placebo-adjusted remission response rate of 10% [6]. There is thus a need to move beyond the monoaminergic model of depressive illness and study the pathophysiological mechanisms of depression within the context of new conceptual frameworks. One such framework that has gained traction over the last several years is the role of the immune system in depression.

Multiple lines of evidence indicate a depression-associated dysregulation of the immune system, particularly innate immunity, leading to an inflammatory-like profile in a proportion of cases with depressive illness. This phenomenon usually is described as "inflammation" in the psychiatric literature, a convention that will be used here with the caveat that evidence for cellular infiltration, the hallmark of inflammation, is minimal. Smith originally proposed the "macrophage theory" of depression which posited that pro-inflammatory cytokines induce depression by affecting hypothalamic function [7] and this theory subsequently was elaborated into the "macrophage-T-lymphocyte" model of depression to emphasize the potentially pathogenic effects of T-cells [8]. In the intervening years empirical findings have largely lent support to these original hypotheses. Specifically, the extant literature is characterized by reports of: (a) elevations of c-reactive protein (CRP) and/or circulating pro-inflammatory cytokines in major depressive disorder (MDD) [9, 10] and bipolar disorder (BD) [11–13], (b) the differential expression of inflammation-related genes in monocytes or peripheral blood mononuclear cells of subjects with mood disorders [14, 15], (c) the anti-inflammatory effect of certain classes of antidepressant medication [16], (d) the epidemiological association between depression and diseases with an autoimmune or inflammatory component [17–19], and (e) the antidepressant effects of anti-inflammatory medications

[20, 21]. While these data are persuasive, there are three issues that are a matter of greater debate. Firstly, it is not yet clear if the associations between inflammatory mediators and depressive disorders are causal. Secondly, the question of whether depression is characterized by central inflammation as well as peripheral inflammation has not yet been answered, and thirdly the mechanisms by which inflammatory compounds putatively cause depressive illness is only partially understood.

Although it is certainly possible that inflammation is simply correlated with depression because of co-occurring medical illnesses or poor lifestyle behaviors (e.g., diet and exercise), there are several strands of research which suggest that in some cases inflammation may play an etiological role in depression. Dantzer and colleagues made the intriguing observation that rodents given lipopolysaccharide (LPS) to induce an inflammatory response, display sickness behavior, an evolutionary adaptation to conserve resources during infection that possesses a striking overlap with classic symptoms of depression such as anhedonia, social withdrawal, anorexia, and sleep disturbances [22–24]. Notably, these depressive behaviors remain present after sickness symptoms have resolved indicating that the effects of inflammation on depressive behaviors (e.g., increased immobility in the forced swim test) are not simply a consequence of decreased motor activity [22]. These experiments cannot be recapitulated in humans, but several groups have taken advantage of "natural" experiments in which non-depressed individuals with hepatitis C or melanoma are treated with interferon alpha (IFNα) or interleukin 2 (IL-2). It has been well established that immune-stimulating treatment of these patients induces a depressive episode in about 30–40% of patients [25–28]. Importantly, there is a temporal disjunction between the "psychological" and "physical" manifestations of this treatment, with the neurovegetative symptoms appearing within 1 week whereas the mood and cognitive symptoms peak 8–12 weeks post-initiation of treatment [29, 30]. Moreover, it is the mood and cognitive symptoms rather than the neurovegetative symptoms that are responsive to antidepressant treatment [29, 30]. Other researchers have administered the typhoid vaccine or low-dose endotoxin to healthy individuals and have demonstrated that a proportion of research participants develop transient mild depressive symptoms [31–33]. Finally, prospective studies have shown a positive association between CRP or interleukin 6 (IL-6) concentrations at baseline and the development of de novo cases of MDD [34, 35] or BD [36].

Regarding the issue of peripheral versus central inflammation, there are *post-mortem* data hinting at parenchymal alterations in immune function (Mechawar and Savitz, in review). Both mRNA and protein levels of TNF, IL-1, and IL-6 have been reported to be significantly increased in the prefrontal cortex (PFC) relative to matched controls [37], while a follow-up study showed that the toll-like receptors: TLR3 and TLR4 were significantly upregulated in the dorsolateral PFC of depressed suicides vs. non-depressed suicides [38]. Consistent with these data, Steiner et al. observed increased densities of HLA-DR-immunoreactive microglial cells in the dorsolateral PFC, anterior cingulate cortex (ACC), and mediodorsal thalamus in suicide victims [39]. Finally, and perhaps most intriguingly, Mechawar and colleagues found that samples from depressed suicides displayed significant

more blood vessels surrounded by a high density of IBA-immunoreactive macrophages than matched controls, conceivably reflecting increased recruitment of circulating bone marrow-derived monocytes in depressed suicides [40].

Although many of the inflammatory mediators discussed above may play an important role in the pathogenesis of depression (see [41, 42] for comprehensive reviews), here we concentrate on a key immuno-regulatory pathway, the kynurenine pathway which is upregulated by the inflammatory response. At least in animal models, two landmark papers showed that kynurenine pathway metabolites appear *necessary* for the induction of depression [43, 44]. That is, LPS does *not* cause depression-like behavior when the activation of the kynurenine pathway is genetically or pharmacologically blocked even when the levels of pro-inflammatory cytokines remain elevated. Thus the kynurenine pathway may be central to the pathogenesis of depressive illness.

2 The Kynurenine Pathway and Its Role in Depressive Illness

Greater than 90% of tryptophan is metabolized into a group of metabolically related compounds termed the "kynurenines" [45] (Fig. 1). The first step in the pathway is the conversation of tryptophan to kynurenine by the enzyme, indoleamine 2,3 deoxygenase (*IDO1*[1]). Pro-inflammatory cytokines, particularly type 1 interferons such as interferon gamma (IFN-γ), but also TNF and CD8+ cells are capable of upregulating *IDO1*, theoretically depriving microorganisms of tryptophan, an essential amino acid that they cannot synthesize [46]. Thus the ratio of kynurenine to tryptophan (KYN/TRP) is standardly used as a measure of *IDO1* activity.

As mentioned above O'Connor and colleagues demonstrated that *IDO1* activity within the brain is necessary for the manifestation of depression-like behavior in mice following intracerebroventricular administration of LPS by showing that the pro-depressive effects of LPS were blocked by genetic deletion or pharmacological inhibition of *IDO1* with 1-methyl-tryptophan (1-MT) [43]. Similarly, Kim et al. demonstrated that the induction of chronic pain in rats induces depressive-like behavior together with *IDO1* upregulation in the hippocampus (but not the thalamus or nucleus accumbens) and plasma via an IL-6 signal transduction pathway. Both the nociceptive and depression-like behavior could be attenuated by *IDO1* gene knockout or pharmacological inhibition of *IDO1* activity [47]. How does *IDO1* activation lead to depressive behavior?

Maes and colleagues initially demonstrated that hepatitis C patients receiving IFNα treatment showed an increase in depressive symptoms and kynurenine

[1] A second isoform of *IDO*, *IDO 2* also exists but less is known about its function. Further, the enzyme, tryptophan-2,3-dioxygenase (TDO) which is produced in the liver, also catalyzes the conversion of tryptophan to kynurenine but is induced by tryptophan and glucocorticoids.

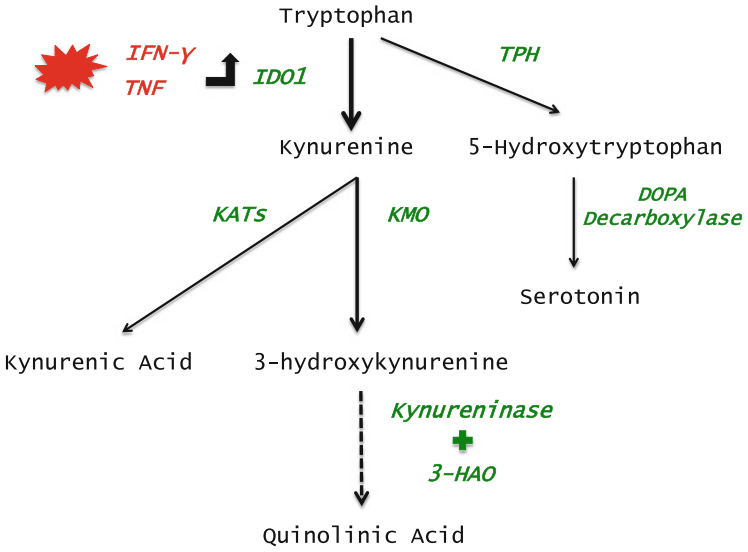

Fig. 1 During the inflammatory response, cytokines such as interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF) upregulate the activity of indoleamine 2,3 deoxygenase (*IDO1*) catalyzing the breakdown of tryptophan into kynurenine at the expense of serotonin. Kynurenine is in turn metabolized along two main branches to form the putatively neuroprotective protein, kynurenic acid, or alternatively, the putatively neurotoxic metabolites 3-hydroxykynurenine and quinolinic acid. Under conditions of inflammation, the breakdown of kynurenine into 3-hydroxykynurenine by the enzyme, kynurenine monooxygenase (*KMO*) is favored over the production of kynurenic acid by the kynurenine aminotransferase (KAT) enzymes. Note that the metabolism pathway has been greatly simplified to illustrate how the main kynurenine metabolites differ from each other

pathway activation along with elevations in the "pro-inflammatory" cytokine, IL-8 [48]. At about the same time, Capuron et al. published a landmark paper showing that systemic administration of IL-2 and IFNα to cancer patients causes a decrease in tryptophan concentration over the course of therapy that is significantly correlated with increases in depressive symptoms [49]. Subsequently, the kynurenine pathway also has been shown to be more activated in medically ill patients with comorbid depression regardless of treatment with immune-modulating mediations [47, 50–52]. With respect to non-medical illness-related depression, Sublette et al. reported an elevation in the peripheral concentration of kynurenine in individuals with a past history of suicide attempts versus depressed individuals without suicide attempts [53]. Partially consistent with these data, a decrease in tryptophan together with an increase in KYN/TRP has been reported in depressed individuals with a history of suicide attempts or active suicidal ideation [54]. A recent meta-analysis also provides evidence for a decrease in peripheral tryptophan levels in depressed individuals although the effect size is small and the decrease in tryptophan could conceivably be related to dietary differences between depressed patients

and healthy participants since not all measures were obtained after overnight fasting [55].

Since tryptophan is actively transported into the brain where it determines the rate of serotonin synthesis, Lapin and Oxenkrug [56] and subsequently Dantzer, Fuchs, Maes, and colleagues [49, 57] proposed that the depressive effects associated with activation of the kynurenine pathway resulted from a reduction in tryptophan levels and by extension serotonin production in the brain. Nevertheless, subsequent research has suggested that serotonin depletion is unlikely to be the mechanism responsible for the depressive effect of inflammatory stimuli. In fact, Dunn and Welch had originally demonstrated that LPS or IL-1 *increases* rather than decreases brain tryptophan and serotonin in mice [58], a result replicated more recently. O'Connor et al. showed that administration of LPS to rodents was found to cause an increase in kynurenine in the brains of mice, but also an increase in brain tryptophan and the turnover of brain serotonin, as measured by the ratio of 5-hydroxy-indoleacetic acid to serotonin [44]. Moreover, the depressive effects of LPS could be blocked with the anti-inflammatory agent, minocycline, or the IDO inhibitor, 1-methyl tryptophan (1MT), without affecting brain tryptophan and serotonin turnover [44]. Additionally, in patients with mood disorders, we consistently have found depression-associated changes in the relative concentrations of downstream kynurenine pathway metabolites in the absence of any significant changes in kynurenine and tryptophan concentrations [59–61] (see below).

If, however, activation of *IDO1* is necessary for the manifestation of the behavioral analogues of depression in preclinical work [43, 44], then what is the mechanism of action since kynurenine is not itself neuroactive? Myint and Kim proposed the "neurodegenerative" model which posited that it is the balance between neurodegenerative and neuroprotective metabolites or more specifically, the relative breakdown of kynurenine into kynurenic acid (KynA) versus 3-hydroxykynurenine (3HK) and its derivatives that is central to the pathogenesis of depressive illness [62].

Kynurenine is metabolized into either KynA by the kynurenine aminotransferase enzymes (*KAT*s) or 3HK by kynurenine monooxygenase (*KMO*); 3HK is in turn metabolized into several neuroactive compounds, including quinolinic acid (QA) (Fig. 1). The KynA and QA branches of the kynurenine pathway are believed to be physiologically separated. Astrocytes, which express the *KAT*s but not *KMO*, produce KynA while 3-HK and its downstream metabolites are synthesized in microglia or macrophages [45, 63, 64]. Possibly because QA in the brain is produced by microglia and infiltrating macrophage cells, under inflammatory conditions, the brain formation of QA predominates over KynA [65, 66]. Given the mutually exclusive breakdown of kynurenine into either KynA or alternatively 3HK and its derivatives, it is not surprising that the metabolites of the two different branches of the kynurenine pathway appear to have distinctive physiological effects.

Although a potentially simplistic model of a complicated biological system, at least *in the context of depressive disorders*, KynA appears to be neuroprotective while 3HK and QA appear to be neurotoxic [60, 67, 68]. KynA is a ionotropic

astrocyte-derived metabolite that acts as an endogenous competitive antagonist of ionotrophic excitatory amino acid receptors including the NMDA receptor where it blocks the glycine co-agonist site [69]. In addition, KynA is an α7 nicotinic receptor *antagonist*, an orphan G-protein-coupled receptor (GPR35) *agonist*, an aryl hydrocarbon receptor (AHR) *agonist*, and an enhancer of nerve growth factor (NGF) expression, potentially regulating the inflammatory response together with glutamatergic, cholinergic, and dopaminergic neurotransmission [70]. Moreover, there are some data to suggest that KynA is an antioxidant and free radical scavenger [71]. Broadly consistent with these data, Schwarcz and colleagues found that *KAT II* knockout mice which received intrastriatal injections of QA developed larger striatal lesions than wild-type animals [72]. Conversely, an elevation in KynA by nicotinylalanine administration produces a dose-related attenuation of QA-induced loss of striatal neurons in rats [73].

In contrast, 3HK is a free radical generator and has been reported to impair mitochondrial function, induce DNA damage and trigger apoptosis [74]. In vitro work has demonstrated that the administration of pharmacological doses of 3HK may kill hippocampal neurons [75] and cortical neurons [76], perhaps explaining why levels of 3HK have been reported to be elevated in the serum of Alzheimer's disease patients [77] and in the brains of Parkinson's disease [78] patients *postmortem*.

QA is a known neurotoxin and gliotoxin [45, 46, 79]. Elevated concentrations of QA have been reported in both the serum and the CSF of patients with neurodegenerative and inflammatory disorders such as Alzheimer's disease and systemic lupus erythematosus patients with neuropsychiatric symptoms [80, 81]. Congruent with the heuristic model of neurotoxic QA-pathway metabolites and neuroprotective KynA [62, 79, 82], pharmacological inhibition of *KMO* is neuroprotective in animal models of cerebral ischemia [83]. The biological mechanisms underlying the neurotoxic effects of QA have been reviewed in detail by Guillemin [46] who describes several potential mechanisms of action that in combination may lead to oxidative stress and neurotoxicity. (1) Activation of NMDA receptors. (2) Potentiation of neuronal glutamate release, inhibition of the reuptake of glutamate by astrocytes, and inhibition of astroglial glutamine synthetase potentially leading to excitotoxicity. (3) Formation of protein complexes with iron leading to the generation of reactive oxygen species and lipid peroxidation. (4) Disruption of the blood brain barrier especially in the region of the striatum and hippocampus. (5) Induction of neuronal nitric oxide synthase leading to increased production of nitric oxide. (6) Phosphorylation of neurofilament subunits and glial fibrillary acidic protein (GFAP) in astrocytes, leading to destabilization of the cellular cytoskeleton. (7) Promotion of tau phosphorylation. (8) Disruption of autophagy. Further, QA has the ability to initiate an inflammatory response or augment existing disease-associated inflammation by enhancing the production of pro-inflammatory proteins [70].

Clearly it is conceivable that neurotoxic kynurenine metabolites such as 3HK and QA may be one pathway through which inflammation affects brain structure and function, leading to depressive illness. However, is there any direct evidence

for this hypothesis? The answer is "yes" although more work needs to be performed to resolve inconsistencies in the literature. As discussed above, the depressogenic effects of IFNα initially were attributed to the activation of *IDO1* and the reduction in tryptophan and serotonin. However, Maes and colleagues first raised the possibility that downstream kynurenine metabolites may be exerting neurotoxic and depressogenic effects. Specifically, Wichers et al. showed that IFNα treatment increased the ratio of plasma kynurenine to KynA was (indicating preferential activation of the QA-pathway) in conjunction with the development of depressive symptoms [84]. Subsequently, Raison et al. reported that both KynA and QA were elevated in the CSF, but not plasma, of IFNα-treated hepatitis C patients although it was the CSF concentrations of QA that were most strongly correlated (positively) with depressive symptoms [85]. Consistent with these data, a more recent study found that the plasma concentrations of QA at 6 and 9 months post-initiation of IFNα treatment for hepatitis C were positively correlated with depression rating scale scores at these times [86]. Georgin-Lavialle et al. recently studied a rare disease called mastocytosis which is characterized by chronic symptoms, including depression, as a result of mast cell accumulation and activation [87]. Compared with controls, patients had lower levels of tryptophan, and increased activity of *IDO1* that were associated with higher perceived stress and depression scores. Importantly, the authors also showed that kynurenine metabolism was preferentially oriented towards QA, with an increase in the QA/KYN ratio in patients that was significantly larger than the increase in the KynA/KYN ratio [87].

Reductions in plasma KynA have been found in patients with primary MDD [68] and depressive-spectrum illness [88], although these studies did not specifically measure metabolites in the QA branch of the pathway and thus no information is available concerning the balance in metabolism between the KynA and QA branches. Our group has, however, consistently found evidence for an imbalance in the synthesis of KynA versus QA-pathway metabolites in depressive illness, i.e., a reduction in serum KynA/3HK and/or KynA/QA in both currently depressed and remitted individuals with MDD [60, 61] and football players with depressive symptoms following concussion [89]. Importantly, we also detected a positive correlation between KynA/QA and the number of months remitted individuals with MDD were depression-free [61], a negative relationship between KynA/QA and symptoms of anhedonia [61], while higher QA and lower KynA/QA at 1-month post-concussion were associated with a worse concussion outcome, defined as number of days until return-to-play [89]. Consistent with our data, a recent study reported persistent decreases in KynA and increases in QA in the cerebrospinal fluid of predominantly depressed subjects up to 2 years after a suicide attempt [67].

Intriguingly, we also have demonstrated that the balance between the KynA pathway versus QA-pathway is associated with brain structure and function in patients with depression. Initially we reported that putative "neuroprotective indices," i.e., the ratio of KynA/3HK and/or KynA/QA was associated with larger hippocampal and/or amygdalar volumes in unmedicated individuals with MDD [60], concussed athletes with symptoms of depression [90], and both unmedicated and medicated individuals with BD [59] (Fig. 2a, b). These data are important

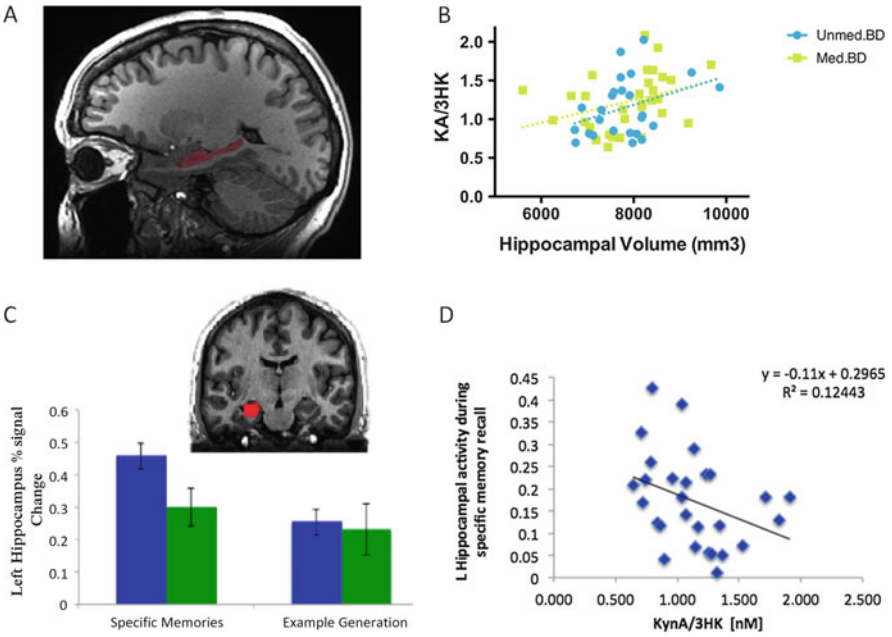

Fig. 2 (**a**) Representative example of the segmentation of the hippocampus by FreeSurfer (*red mask*) shown in the sagittal plane. (**b**) Scatterplots showing the correlation between KynA/3HK and the volume of the hippocampus in the unmedicated (*blue circles*) and medicated (*yellow squares*) participants with bipolar depression. (**c**) Coronal anatomical MRI slice showing the left hippocampal mask overlay and the percent signal change in the left hippocampus during specific autobiographical memory recall and example generation in the MDD (*blue*) and healthy control (*green*) groups. (**d**) Scatterplot showing the correlation between KynA/3HK and activity of the left hippocampus during specific autobiographical memory recall in the MDD group

because a reduction in hippocampal volume, which is thought to be caused by dendritic atrophy [91–93], is one of the best-replicated findings in biological psychiatry [94–97]. Further, in a follow-up study we replicated previous work showing that depressed patients were less successful at retrieving autobiographical memories than controls and that this deficit in performance was associated with increased left hippocampal activity (indicating more effortful processing) during the recall of positive and negative memories (Fig. 2c). Importantly, we found that KynA/3HK was *inversely* associated with left hippocampal activity during specific autobiographical memory recall in participants with MDD (Fig. 2d), indicating that a greater "neuroprotective index" was associated with better hippocampal function (Young et al. in review). Our finding also relates to the preclinical work of Heisler and O'Connor who demonstrated that mice deficient in *IDO1* or *KMO* were protected from endotoxin-induced deficits in novel object recognition, a task that is dependent on normal function of the hippocampus [98]. Our data thus suggest that an elevation of neurotoxic metabolites might be one possible source of the hippocampal pathology and cognitive deficits associated with depression.

This pathophysiological relationship also may extend to other regions of the brain. We recently showed that compared with healthy controls, MDD patients showed a reduction in cortical thickness of the right medial PFC (BA24 and BA32), and further, that both KynA/3HK and KynA/QA ratios at least partially mediated the relationship between diagnosis and cortical thickness of the right BA32 [99]. This finding is consistent with a *postmortem* immunohistochemistry study showing that a mixed sample of MDD and BD subjects had increased QA-positive cell densities in the anterior mid-cingulate cortex and subgenual ACC, suggesting microglial cell activation in these regions of the PFC [100].

In the case of bipolar depression, specifically, the findings are more variable. Similar to our findings in MDD, we reported reductions in serum KynA/QA and KynA/3HK in both unmedicated and medicated depressed subjects with bipolar depression [101]. Partially consistent with our data, Myint and colleagues [102] reported a reduction in plasma KynA levels in manic patients relative to healthy controls while an ex vivo study of skin fibroblasts derived from bipolar depression subjects found disproportionate elevations in 3HK relative to KynA after stimulation with pro-inflammatory cytokines [103]. Further, in 23 patients with either MDD or BD treated twice weekly with electroconvulsive therapy (ECT) over 6 weeks, KYN/TRP, KynA, and KynA/3HK increased over time compared to baseline [104]. This finding raises the possibility that a shift in balance towards molecules such as KynA, with potential neuroprotective properties may mediate the antidepressant effects of ECT. In contrast, Olsson et al. reported that KynA concentrations in the CSF of medicated, euthymic males with BD were elevated versus healthy controls [105], and the elevation in KynA was associated with a history of psychotic episodes [106]. In addition, Lavebratt et al. [107] found a reduction in the *postmortem* expression of the enzyme, *KMO* (implying a possible increase in KynA versus 3HK) in the dorsolateral prefrontal cortices of BD patients with a history of psychosis.

Given the data that QA impairs astrocyte function and glutamate recycling [46], it may be relevant that *postmortem* studies examining the expression of the astrocyte-specific intermediate filament GFAP have consistently reported it to be significantly decreased in depressed cases versus controls in the PFC [108–110]. Moreover, other astrocyte-specific genes such as the tropomyosin-related kinase B receptor (TrkB.1) isoform [111] and connexins 43 and 30 [112, 113] also are significantly downregulated in the PFC of suicide completers. In addition, decreased glutamine synthetase expression has been reported in the amygdala and PFC of suicide completers [114, 115]. Glutamate is converted into glutamine by glutamine synthetase and glutamine is in turn delivered to neurons where it is re-converted into glutamate. Thus evidence of decreased expression of glutamine synthetase in *postmortem* samples from individuals with a history of depression, potentially, is consistent with elevations of QA in the brains of these individuals.

These data together with the opposing effects of KynA and QA on the NMDA receptor raise the possibility that activation of the kynurenine pathway ultimately affects depressive symptoms and behavior by altering glutamatergic rather than serotonergic neurotransmission as originally believed [116, 117]. Direct evidence

for this hypothesis was provided by Dantzer and colleagues. Mice given LPS showed significant increases in 3HK, 3-hydroxyanthranilic acid (a metabolite of 3HK), and QA (but not KynA) in the brain along with sickness and depression-like behavior [66]. Pretreatment with the NMDA antagonist, ketamine, had no effect on sickness behavior, inflammatory cytokines or kynurenine metabolites, but blocked the LPS-induced depression-like behavior [66]. In humans, Haroon, Miller, and colleagues have published several papers demonstrating that inflammation is associated with increased glutamate in the context of depression. Using a quasi-experimental design, this group employed single-voxel magnetic resonance spectroscopy (MRS) to measure glutamate concentrations normalized to creatine (Glu/Cr) in the dorsal ACC (dACC) and basal ganglia of hepatitis C patients before and after either no treatment or treatment with IFNα [118]. Treatment with IFNα led to an increase in Glu/Cr in the dACC and left (but not right) basal ganglia and the change in Glu/Cr in the left basal ganglia was associated with a decrease in motivation [118]. In a follow-up, cross-sectional study of unmedicated patients with major depression, the authors showed a positive correlation between Glu/Cr in the left basal ganglia (but not dACC) and plasma CRP concentrations [119]. Unfortunately, kynurenine pathway metabolites were not analyzed in these studies and thus it is unclear if the inflammation-associated changes in glutamate were associated with activation of the kynurenine pathway.

3 Conclusion

Depression is a pathophysiologically and etiologically heterogeneous disorder. At least a subgroup of individuals – perhaps on the order of 30% of patients [120] – show evidence of inflammation, and preclinical, quasi-experimental, and longitudinal studies suggest that inflammatory processes may play a causal role in the developmental of depressive illness. While there may be multiple causal pathways through which inflammatory processes affect mood, the animal literature suggests that activation of the kynurenine pathway is essential for the development of depression-like behavior in rodents. Studies of hepatitis C or cancer patients receiving treatment with IFNα or IL-2 show increased activity of *IDO1* and decreased levels of tryptophan that appear to correlate with inflammation-induced depression. Further, this treatment has been shown to lead to preferential activation of the QA-pathway over the KynA pathway. Similarly, in non-medically ill patients with major depression, multiple studies have found activation of the kynurenine pathway and/or preferential activation of the QA-pathway. Initially, activation of the kynurenine pathway was believed to precipitate depressive symptoms by depleting serotonin, however, the weight of the evidence now suggests that an imbalance between neurotoxic and neuroprotective metabolites may be the principal driver of depression; conceivably via its effects on glutamatergic neurotransmission.

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Suicidality and Activation of the Kynurenine Pathway of Tryptophan Metabolism

Elena Y. Bryleva and Lena Brundin

Abstract A recent report by the World Health Organization declared suicide to be a major global problem. With more than 800,000 lives lost each year, suicide is calculated to be the 14th leading cause of death around the world. While the biological mechanisms causing suicidal ideation and behavior are not fully understood, increased levels of inflammation, arising from various sources, have been detected in the central nervous system and the peripheral blood of suicidal patients and suicide completers. Inflammation induces the kynurenine pathway of tryptophan metabolism, which generates a range of metabolites with potent effects on neurotransmitter systems as well as on inflammation. Recent evidence indicates that a dysregulation of the enzymes in the kynurenine pathway may be present in suicidal patients, with a resulting imbalance of metabolites that modulate glutamate neurotransmission and neuroinflammation. As the body of research in these areas grows, targeting the kynurenine pathway enzymes and metabolites may provide novel therapeutic opportunities for detection, treatment, and ultimately prevention of suicidal behavior.

Keywords 3-Hydroxyanthranilic acid • Cytokine • Depression • Glutamate • Inflammation • Kynurenic acid • Kynurenine pathway • Picolinic acid • Quinolinic acid

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1 Introduction

It is estimated that every 40 s suicide claims a life somewhere in the world, amounting to over 800,000 deaths annually [1]. There are indications that the actual number of deaths by suicide is actually higher than reported, due to under-reporting in countries where suicide is stigmatized and illegal [2]. Suicide is the 14th cause of death around the world [3] and tragically the 2nd leading cause of death in young population (15–29 years of age) [1]. There are indications that for each completed suicide 10–20 suicide attempts take place. Suicide was recently declared to be a major global public health problem by the World Health Organization as it causes profound psychological, social and economic suffering to individuals, families, and countries [1]. Every year in the USA, suicide results in a $44 billion loss to the economy in medical expenses and lost productivity and each prevented suicide is calculated to save an average of over $1,160,000 [4].

Suicidality is a cross-diagnostic phenomenon with biological underpinnings likely to be shared across different disease states. Still, these common biological underpinnings remain to be fully clarified. Since around 90% of suicide completers suffer from a psychiatric illness, treatment of suicidality is currently largely guided by the primary psychiatric diagnosis [5]. Theoretically, suicide is considered to be largely preventable as there are many available clinical treatment choices and interventions; however, the suicide rate is still increasing in many countries [1]. This could in part be due to the lack of an accurate risk assessment by the health care system, as almost half of the suicidal patients contact their primary care or mental health provider in the month prior to the suicide [6, 7]. Therefore, it is of critical importance to refine the methods of suicide risk detection.

The treatment interventions of patients with suicidal ideation and behavior often consist of anxiolytics and antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs). Even though SSRIs and SNRIs are usually the first-choice antidepressant treatments, their usefulness is limited by the fact that it can take weeks to develop positive mood effects and up to 50% of patients do not benefit from them even after

months of usage [8]. Furthermore, during the first two weeks of treatment these treatments can increase the risk of suicide, predominantly in children and youth [5]. Other pharmacological agents capable of decreasing suicidal behavior (attempts and suicide completion) include electroconvulsive therapy in treatment-resistant depression [9], clozapine in schizophrenia [10], and lithium in patients with major affective disorders [11, 12]. A couple of years ago, clinical trials found that an intravenous injection of an NMDA-receptor antagonist ketamine can generate a strong antidepressant and anti-suicidal effect within hours [13, 14]. Currently, more research is needed to uncover the biological mechanisms underlying the beneficial effects of the above-mentioned treatments.

2 Risk Factors of Suicide

The best predictor of a future death by suicide is a history of previous attempts [15]. Mounting evidence suggests that the intricate interactions between genetic and environmental variables are likely responsible for manifestation of suicidal behavior [16]. Findings from epidemiological studies have concluded that heritable factors may explain around 40% of suicidal behavior [17]. These factors include, but are not limited to, variants of the serotonin transporter gene (5-HTT) [18] and the tryptophan hydroxylase 1 gene (TPH1) [19]. Recently, it has been proposed that some of the interactions between genes and environment could be modulated by epigenetic factors and lead to certain changes, such as hypermethylation of the brain-derived neurotrophic factor (BDNF) promoter [20, 21], which are important in depression and suicidality [22]. Having a psychiatric disorder, especially major depressive disorder (MDD) or bipolar disorder, is another risk factor of suicide as 90% of suicide completers suffer from some form of psychiatric illness [23]. In addition, exhibiting specific personality traits, such as hopelessness [24] or impulsivity and aggression, especially in youth [25, 26], serve as additional factors regardless of the presence or absence of the psychiatric illness. Males tend to be at a higher risk of completing suicide than females as the male:female ratio of global age-standardized suicide rate is 1.9 [1].

While the biological changes that may give rise to suicidal ideation and behavior are not completely understood, increased levels of inflammation, arising from various sources, have been detected in the central nervous system (CNS) and the peripheral blood of suicide patients. Some inflammatory mediators, such as cytokines, are able to reciprocally interact with and are thought to be partially responsible for the dysregulations of the hypothalamic-pituitary-adrenal (HPA) axis [27] and serotonergic system [28] frequently observed in suicidal patients. In addition, inflammation causes activation of the kynurenine pathway of tryptophan (TRP) degradation and potentially leads to its dysregulation, the effect of which is detected in suicidal patients as the imbalance of the pathway metabolites. As some of these metabolites modulate glutamate neurotransmission and neuroinflammation they may directly contribute to manifestation of suicidal symptoms.

3 Activation of the Kynurenine Pathway

Around 90% of the dietary TRP is degraded through the kynurenine pathway [29] and enzymes of this pathway have been found in many tissues and cells, including brain, liver, intestine, and cells of the immune system. The first step of this pathway is initiated by indoleamine 2,3-dioxygenase 1 (IDO1), IDO2, or tryptophan 2,3-dioxygenase (TDO) to produce *N*-formylkynurenine, which subsequently gets converted to kynurenine (KYN) (Fig. 1). The expression levels of IDO1, IDO2, and TDO enzymes are much lower in the brain than in the peripheral organs

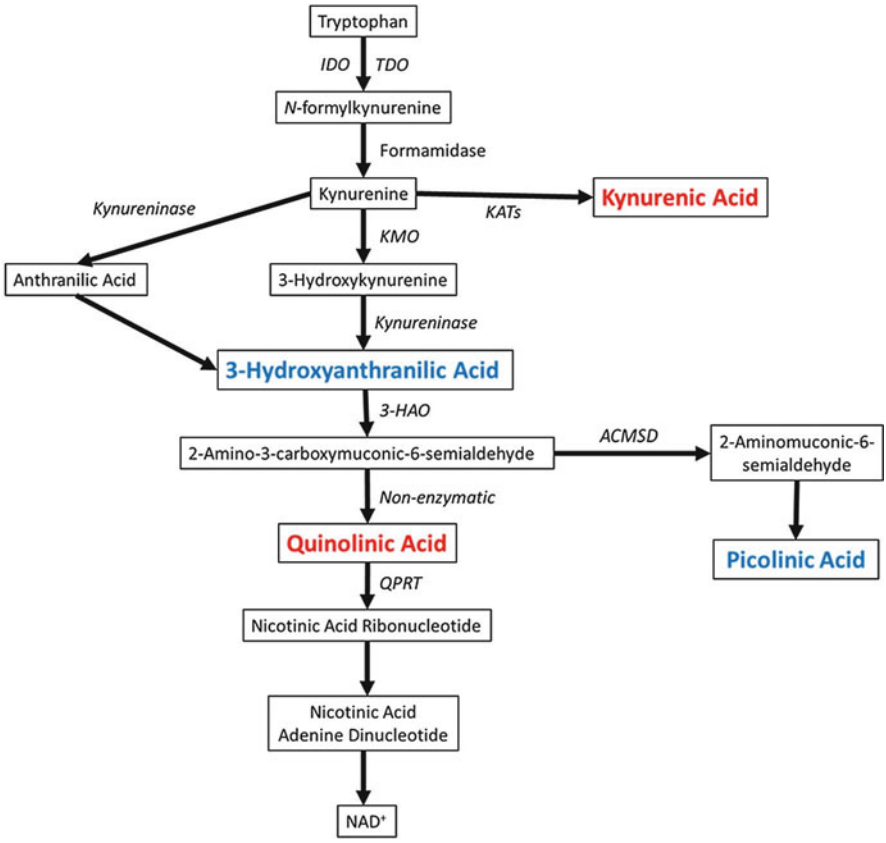

Fig. 1 The kynurenine pathway. The pathway enzymes are in *italics* and metabolites are *boxed*. The metabolites, for which there is accumulating evidence supporting a role in suicidality, are highlighted in *red* and the metabolites, for which data is more sparse, are highlighted in *blue*. *IDO* indoleamine 2,3-dioxygenase, *TDO* tryptophan 2,3-dioxygenase, *KATs* kynurenine aminotransferases, *KMO* kynurenine-3-monooxygenase, *3-HAO* 3-hydroxyanthranilate-3,4-dioxygenase, *ACMSD* 2-amino-3-carboxymuconic-6-semialdehyde decarboxylase, *QPRT* quinolinate phosphoribosyltransferase, *NAD* nicotinamide adenine dinucleotide

[30]. Consequently, around 60% of the kynurenine pathway metabolism in the brain estimated to be initiated by the first metabolite KYN, which readily crosses the blood brain barrier (BBB) and enters the CNS from the periphery [31]. KYN is then further metabolized to produce several neuroactive intermediates potentially important in the generation of depressive and suicidal symptoms, including quinolinic acid (QUIN), kynurenic acid (KYNA), picolinic acid (PIC), and 3-hydroxyanthranilic acid (3-HAA) (Fig. 1) [32]. The kynurenine pathway can be stimulated by the increased levels of pro-inflammatory cytokines, such as interferon-γ (IFN-γ), interleukin-1β (IL-1β), and IL-6, all of which are inducers of IDO1 and TDO [33, 34]. In addition, environmental stresses may activate the kynurenine pathway via the induction of TDO by cortisol [35]. Besides being the first substrate in the kynurenine pathway, TRP is also a precursor for neurotransmitter serotonin. Consequently, activation of the kynurenine pathway may decrease the available TRP for serotonin synthesis [36] and may be in part responsible for the observed low levels of 5-hydroxyindoleacetic acid, a main metabolite of serotonin, in the cerebrospinal fluid (CSF) of suicide attempters [37]. However, the hypothesis that induction of the kynurenine pathway contributes to a decrease in serotonin levels in the brains of suicidal patients has not been fully proven.

4 Kynurenine Pathway Metabolites in Psychiatric Disorders and Suicidal Behavior

Dysregulation of the kynurenine pathway was reported in suicidal patients for the first time in 2011 [38]. This study found significantly increased levels of plasma KYN in previous suicide attempters compared to depressed patients without history of previous attempts, indicating an increase in TRP metabolism through kynurenine pathway. In agreement with this, a later study by Bradley et al. detected an approximately 40% decrease in plasma TRP and an increase of 40% in KYN/TRP ratio in suicidal youth compared to ones with MDD but no suicidality and healthy controls [39]. Both KYN and TRP are able to cross the BBB and enter the brain from circulation by active transport [32]. Once in the brain KYN will be taken up by glial cells and processed through different arms of the pathway. In microglial cells and cells of monocytic origin, KYN is processed to produce 3-hydroxykynurenine (3-HK) by the enzyme kynurenine-3-monooxygenase (KMO) (Fig. 1) [40]. 3-HK gets subsequently converted into metabolites which include 3-HAA and QUIN. On the other hand, in astrocytes, which lack KMO, KYN is instead metabolized into KYNA (Fig. 1) [41]. In addition to astrocytes, neurons and oligodendrocytes are also capable of generating KYNA [42, 43].

4.1 Quinolinic Acid

The non-enzymatic synthesis of quinolinic acid (QUIN), which is considered to be one of the most important bioactive metabolites of the kynurenine pathway, occurs from a precursor 2-amino-3-carboxymuconic-6-semialdehyde (ACMS) (Fig. 1). The spontaneous conversion to QUIN takes place when a competing enzyme 2-amino-3-carboxymuconic-6-semialdehyde decarboxylase (ACMSD), which metabolizes ACMS into PIC, is saturated, inactive, or absent. Since QUIN does not readily cross the BBB [44], it is locally formed in the brain, primarily by microglia and infiltrating macrophages, and is considered to be largely neurotoxic through several mechanisms [41, 45, 46]. First, QUIN is an agonist of the glutamate *N*-methyl-D-aspartic acid receptors (NMDARs) and has strong affinity for NMDARs that contain NR1+NR2A and the NR1+NR2B subunits. Since the expression of these subunits is primarily limited to the forebrain [47], neurons in the hippocampus, striatum, and neocortex are most sensitive to QUIN toxicity while neurons in the spinal cord and cerebellum are least affected by it [48]. Suicide committers, especially females, have a higher expression of these NMDAR subunits in dorsolateral prefrontal cortices compared to controls [49]. This raises a possibility that patients with increased expression of these NMDARs might be more sensitive to elevated levels of their agonist QUIN. QUIN also exerts its toxicity by stimulating the neuronal glutamate release and promoting its accumulation by inhibiting its uptake and breakdown by astrocytes [50]. The resulting increase in extracellular glutamate levels overstimulates the glutamatergic system and leads to neuronal death. Through forming complexes with iron ions, QUIN also increases lipid peroxidation and promotes formation of reactive oxygen species [51, 52].

In 2013 we initiated a study to investigate the relationship between QUIN and suicidal behavior [53]. We became interested in this topic due to the findings in clinical trials that reported a rapid and long-lasting anti-suicidal effect of the anesthetic compound ketamine [54–57]. Ketamine, which is an NMDAR antagonist, is capable of decreasing suicidal ideation as early as 40 min post-infusion and this effect can last for up to 10 days [54, 55, 57, 58]. The anti-depressive and anti-suicidal effects of ketamine were later confirmed by other clinical studies [59]. Furthermore, a study by Walker et al. reported that ketamine blocks the depression-like behavior of mice treated with lipopolysaccharide (LPS) [60]. Intraperitoneal injection of LPS, which is a widely used method to induce depression-like phenotype in animal models, increases inflammation in the brain [61] and, thus, induces IDO which can lead to an increased synthesis of QUIN in the mouse brain [60, 62]. The study by Walker et al. demonstrated that depression in this animal model, as induced by the LPS injection, could be reversed specifically by the actions of ketamine on the NMDA receptor [60]. The results of the human and animal studies using ketamine suggest that hyperstimulation of NMDAR may, at least in part, be responsible for the generation of depressive and suicidal behavior, although ketamine additionally displays other beneficial effects in the brain [63]. Even though these studies did not attempt to measure QUIN, the results point to this kynurenine

metabolite being of specific interest as a potential biological cause of the NMDAR hyperstimulation.

In the initial study analyzing the association between QUIN and suicidality, we detected a 300% increase in the CSF level of QUIN in suicide attempters compared to healthy controls [53]. QUIN also positively correlated with suicidal intent, measured by the Suicide Intent Scale [64], and with CSF IL-6 levels, suggesting that an increase in inflammatory process stimulates formation of QUIN, which then promotes symptoms of suicidality. Following a suicide attempt, QUIN levels slightly decreased but were still approximately to 150% compared to QUIN levels in healthy controls over a 2-year period [65]. Elevation of QUIN in suicidal attempters could potentially also contribute to the development of structural deficits observed in such patients [66], as high levels would induce not only increased glutamate neurotransmission but also contribute to excito- and neurotoxicity with cell death as a consequence. Certain cortical regions (such as subgenual anterior cingulate cortex (sACC) and anterior midcingulate cortex (aMCC)) in brains of depressed individuals who died by suicide have been shown to contain an increased density of QUIN-positive microglial cells [67]. However, there are some region-specific differences in the distribution of the QUIN-containing microglia since its density was decreased in hippocampi of the same suicide committers [68].

4.2 Kynurenic Acid

Of the kynurenine pathway metabolites, kynurenic acid (KYNA) was the first one discovered and characterized. Kynurenine aminotransferase enzymes convert KYN into KYNA through transamination reaction (Fig. 1). Since KYNA crosses the BBB very poorly under physiological conditions [44], most of the KYNA in the brain is locally produced by neurons and certain glial cells such as astrocytes and oligodendrocytes [41–43]. As opposed to QUIN, KYNA is an antagonist of a variety of ionotropic glutamate receptors which include NMDARs, AMPA receptors (AMPARs), and kainite receptors. More recent studies found that KYNA is also capable of inhibiting α7 nicotinic acetylcholine receptor (α7nAChR) and activation of the aryl hydrocarbon receptor [69]. In addition, KYNA bind and activates an orphan G protein-coupled receptor GPR35 which is thought to reduce the extracellular glutamate levels in the brain, as well as prevent the release of pro-inflammatory cytokines by monocytes and macrophages [70, 71]. Besides its role in receptor binding, KYNA also possesses anti-oxidant properties and is able to scavenge free radicals, including superoxide anion and hydroxyl radical [72, 73].

Even though the above-mentioned properties indicated that physiological levels of KYNA are largely neuroprotective and anticonvulsive, increased levels of KYNA have been associated with psychosis and cognitive deficits [74]. Indeed, the CSF KYNA levels in schizophrenia spectrum disorder patients are approximately 50–70% higher than in healthy subjects [75, 76]. However, schizophrenia patients who attempted suicide display significantly lower levels of CSF KYNA

compared to non-suicidal patients [77]. We have recently found that over a period of 2 years following a suicide attempt, the CSF KYNA levels decreased by approximately 35% and the low levels were associated with more severe psychiatric symptoms, including suicidal ideation and depression severity [65]. Because KYNA and QUIN both interact with NMDAR and promote either its activation or inhibition, respectively, the QUIN/KYNA ratio has been used in the literature to represent the overall degree of NMDAR stimulation. This ratio is often referred to as the neurotoxic ratio even though prior to any neurotoxic outcome, the effects of QUIN and KYNA on the NMDAR are antagonistic/agonistic. Suicide attempters display a more than twofold increase in CSF QUIN/KYNA ratio compared to healthy controls, suggesting an increase in net positive NMDAR stimulation in suicidality [53].

One of the limitations in investigating the specific etiology of suicidal behavior is the fact that suicidal patients are frequently either excluded from the clinical studies on depressed patients or are being pooled together with non-suicidal patients without specifically accounting for the presence of suicidality. Similar to our findings in suicide attempters, a study by Myint et al. detected a 32% decrease of KYNA levels in peripheral blood from a depressed population, of which almost 20% had previously attempted suicide [78]. QUIN was not measured in this study. A more recent study found a significant decrease of neuroprotective KYNA/QUIN ratio in serum from patients with both current and remitted MDD compared to healthy controls, which indicates that patients with MDD display a persistent imbalance of the kynurenine pathway even in the absence of current depressive symptomology [79]. Both MDD groups in this study included previous suicide attempters.

4.3 Picolinic Acid

As mentioned in Sect. 4.1, picolinic acid (PIC) is produced by the ACMSD enzyme from the unstable metabolite ACMS (Fig. 1). We and others have previously detected PIC in human CSF samples (our unpublished observations) [80, 81]. However, whether PIC is primarily synthesized in the CNS or whether there is an additional contribution from the periphery is currently unknown, as PIC's ability to cross the BBB under normal physiological conditions has not been determined. The ACMSD enzyme is expressed in the brain, albeit at much lower levels compared to kidney and liver [82], and, judging from animal models, its expression and activity in CNS can be reduced or induced under conditions such as low protein diet or streptozocin-induced diabetes, respectively [83]. In the brain, ACMSD expression has been found in neuronal and glial cells of the hippocampus and cortex, and PIC production has been detected in primary cultures of fetal human neurons [84]. As PIC cannot be further degraded, it is considered to be an end-product of the kynurenine pathway and is excreted through bile and urine. PIC is, perhaps, best known for its ability to chelate metal ions, including copper

and iron [85]. Intriguingly, PIC is able to block the neurotoxic properties of QUIN in cell culture and animal models, possibly by chelation of the endogenous zinc [86–89].

We have found that the PIC levels in blood and CSF were consistently decreased among several different cohorts of suicide attempters (manuscript in submission). This may indicate that the activity of the ACMSD enzyme is reduced in suicidal patients, which is likely to contribute to the observed increase in the levels of QUIN and, consequently, neuroinflammation found in these patients. PIC is able to bind and chelate chromium and the resulting chromium picolinate complex produces a reduction of depressive symptoms in atypical depression [90, 91] and increases the efficacy of pharmaceutical drugs for dysthymic disorder [92]. Chromium picolinate also induces antianxiety and antidepressant effects, most likely through the observed decreases in plasma corticosterone levels and increases in the cortical and cerebellar serotonin levels in animal models of chronic unpredictable mild stress [93]. It is often presumed that in the chromium picolinate complex, PIC is the non-active component while chromium is the one responsible for the observed favorable effects. However, based on the in vitro and animal studies that show PIC is able to protect against neurotoxic effects of QUIN as well our own study in which decreased levels of PIC were observed in suicide attempters, it is possible the PIC in chromium picolinate complex might play an active role in decreasing the depressive symptoms.

4.4 3-Hydroxyanthranilic Acid

3-hydroxyanthranilic acid (3-HAA) is produced by kynureninase and does not readily cross the BBB (Fig. 1) [44]. Thus, 3-HAA presence in the brain is due to its local synthesis primarily by microglia and cells of monocytic origin [32]. The substrate preference for the kynureninase enzyme varies depending on the tissue type and while in the brain anthranilic acid is the preferred precursor for 3-HAA, 3-HK is the primary precursor in the periphery [94]. 3-HAA is a highly redox-active compound [95], which, depending on the local redox conditions, can possess either anti-oxidant [96] or pro-oxidant [97] properties.

Recently, the connection between 3-HAA levels in peripheral blood and suicidality was explored. However, no significant differences in the plasma levels of 3-HAA were detected between depressed youth with suicidal behavior ($N = 20$), depressed adolescents without suicidal behavior ($N = 30$), and healthy controls ($N = 22$) [39]. Likewise, plasma 3-HAA levels (as well as kynureninase enzyme expression) remained the same between MDD patients and healthy controls [98]. Interestingly, when studies investigating adolescents with MDD stratified the patients into groups with or without melancholic features, only the MDD group with melancholic features (unlike MDD without melancholic features or healthy controls) presented a positive association between plasma 3-HAA/KYN and severity of MDD episode [99, 100]. The youth group with melancholic-type

MDD also had a positive correlation between the plasma 3-HAA levels and striatal total choline, which is a biomarker for cell membrane breakdown [100]. The results of these studies indicate that, at least in adolescence, melancholic-type MDD possesses certain neurobiological features, which distinguish it from other subtypes of MDD.

5 Conclusions

An increasing number of studies indicate that there is a dysregulation of the kynurenine pathway enzymes in suicidal patients. The resulting concentration imbalance of key bioactive metabolites, such as QUIN, KYNA, PIC, and 3-HAA, is thought to be important in generation of psychiatric symptoms. One of the possible biological mechanisms behind generation of suicidal behavior could be increase in the levels of QUIN, which, by being an NMDAR agonist, causes overstimulation of the glutamatergic system and neurotoxicity. Ketamine, which in an NMDAR antagonist, produces a rapid anti-suicidal and antidepressant effect, possibly by antagonizing NMDARs and decreasing the hyperstimulation of the glutamate neurotransmission produced by QUIN.

In the future it will be important to uncover the mechanisms underpinning the dysregulation of the kynurenine pathway enzymes, and to understand the conditions that allow accumulation of certain metabolites. It is likely that the activity and expression of the enzymes could be, at least in part, affected by variations in the corresponding genes [101]. It will also be important to discover what predisposition factors produce resilience or vulnerability to manifestation of suicidal behavior. It is known that certain pro-inflammatory cytokines, found to be increased in patients with suicidality, activate the initial enzymes of the kynurenine pathway. However, to what extent these cytokines influence the subsequent enzymes of the pathway is currently not fully established. As the body of literature investigating the relationship between kynurenine pathway regulation and suicidality grows, opportunities for therapeutic intervention through manipulation of the enzyme activity may become apparent. Moreover, measuring circulating metabolites could provide a future way of improving suicide risk detection in the clinic.

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The Promise and Limitations of Anti-Inflammatory Agents for the Treatment of Major Depressive Disorder

Charles L. Raison

Abstract This review provides a critical perspective on recent meta-analyses suggesting that several anti-inflammatory modalities, including nonsteroidal anti-inflammatory drugs (NSAIDs), omega-3 fatty acids, and cytokine antagonist, possess generalizable antidepressant properties. By examining confounds and limitations in the available literature it is suggested that current data suggest that only a sub-group of individuals with major depressive disorder (MDD) have evidence of increased inflammatory biomarkers and it is in these individuals that anti-inflammatory agents show promise for reducing depressive symptoms. The treatment implications of this cautionary perspective are discussed.

Keywords Antidepressants • Cytokine antagonists • Cytokines • Inflammation • Major depressive disorder • Nonsteroidal anti-inflammatory drugs • Omega-3 fatty acids

Contents

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1 Introduction

What do hula hoops, the dexamethasone suppression test, country western dancing, lobotomies, cabbage patch kids, and gabapentin for the treatment of mania have in common? They are all fads, phenomena that swept through their respective cultures with the intensity of a forest fire, only to fade as quickly and almost as completely as they had come. And while cultural fads generally come and go without inflicting much long-term damage, we have been less lucky in the field of mental health research.

For us, fads have caused mischief for at least two reasons. First, the hope for a "magic bullet" that would provide clarity to our diagnostic quandaries and power to our treatments has caused us to prematurely implement interventions that were either ineffective or damaging. Second, the overly enthusiastic embrace of complex scientific findings has led us, time and again, to prematurely abandon these same findings when they failed to deliver on our unrealistic expectations of them.

Thus, rather than filling me with satisfaction, as one who has long researched links between inflammation and brain function, I fear our current romance with the notion that major depression is an inflammatory condition to be treated with anti-inflammatories. I fear it because I suspect that valuable scientific insights associating the immune system with major depression (MDD) will be down-graded and dismissed when they fail to deliver the type of definitive treatments that we so desperately need. And I fear it because available data suggest that – when taken as a whole – patients with depression may be as likely to be hurt as helped by a well-meaning blanket application of anti-inflammatory modalities to assuage these individuals' symptoms.

In this paper I provide a critical review of the notion that MDD is an inflammatory condition and that anti-inflammatory agents hold potential as "all-purpose" antidepressants. This perspective is at odds with recent meta-analyses suggesting that MDD is associated with increased inflammation and that – taken as a whole – anti-inflammatory agents produce antidepressant effects. I will attempt to show that while true, these conclusions mask the fact that positive findings result from heterogeneity within the respective datasets. Said more simply, I suggest that inflammation likely contributes to the development and maintenance of depression in only some individuals and that it is these individuals who may well benefit from pharmacologic interventions that inhibit inflammatory activity. Disturbingly, I will provide some evidence that anti-inflammatory strategies may actually harm some patients with MDD.

2 Is Depression an Inflammatory Condition and Does It Matter?

By way of a thought experiment, suppose a middle-aged patient complaining of severe depression comes to your office for treatment. Believing that MDD is an inflammatory condition, you measure plasma concentrations of inflammatory cytokines and the acute phase reactant c-reactive protein (CRP). For extra measure, you perform a lumber puncture in your office to measure cerebrospinal fluid concentrations of the same inflammatory markers. A few days later the patient returns, and you hold the lab results in your hands. No evidence of increased inflammation in either the central nervous system (CNS) or periphery. The patient is weeping, can't sleep, doesn't want to eat, is exhausted, and says he can think of nothing but killing himself. Would you decide that because the patient's inflammatory measures are normal he cannot be depressed?

In fact this situation is far from hypothetical. While numerous studies indicate that inflammatory biomarkers (especially interleukin [IL]-6, IL-1-beta and tumor necrosis factor [TNF]) are elevated in groups of depressed individuals compared to groups of non-depressed individuals [1–5], many severely depressed patients have low levels of inflammation. Indeed, in study after study the values for any given inflammatory marker overlap between groups of depressed and non-depressed individuals, regardless of how much higher the marker's mean value may be in the depressed group. This means that a large proportion of the depressed group in any given study has values similar to the non-depressed group, and always in the "normal" range for the marker in question [6–11], when such a norm has been established [12]. Given this, in what way can MDD be conceived of as an inflammatory condition, and why would we expect an anti-inflammatory treatment to benefit patients without evidence of increased inflammation?

In fact, there is a sense in which MDD could be an inflammatory condition even in those individuals demonstrating low levels of inflammatory biomarkers, just as we recognize depression as linked to the functioning of norepinephrine and serotonin, even though a majority of depressed patients do not show measurable abnormalities in these neurotransmitters [13]. As with monoamine neurotransmitters, inflammatory pathways do not exist in functional isolation from other physiological systems in the body that have been implicated in the pathogenesis of MDD, but rather have been shown repeatedly to interact these systems in ways known to promote depression [14–17]. As a result of response differences in these systems people appear to vary widely in their sensitivity to the behavioral effects of inflammatory signaling. For example, women have appeared to be more likely than men to develop depressive symptoms in response to a dose of lipopolysaccharide (LPS) [18], and a variety of pre-treatment behavioral and biological factors have been shown to increase the risk of depression in response to chronic inflammatory stimulation induced by therapy with interferon (IFN)-alpha [19–23]. Thus, some individuals' overall physiology might protect them from developing depression in response to all but the highest levels of inflammatory stimulation; whereas

the physiology of others might make them prone to developing depressive symptoms in response to even low levels of inflammatory stimulation.

These considerations suggest that MDD might be an inflammatory condition as a result of at least two semi-independent processes. First, inflammation might directly contribute to MDD in the sub-group of patients with chronically elevated inflammation. Second, inflammation might contribute indirectly to the development/maintenance of MDD in a larger group of depressed individuals with normal levels of inflammation, but who demonstrate increased sensitivity to the depressogenic effects of inflammatory signaling.

This scenario lends itself to two strong predictions. First, modalities that reduce inflammatory cytokine activity should produce an antidepressant effect that is observable in patients with both elevated and normal levels of inflammatory biomarkers. And second, because a wide range of systems may contribute to the downstream antidepressant effects of anti-inflammatory agents, one would not expect to see a clear association between baseline inflammatory levels and subsequent antidepressant response.

Alas for those of us who would like to view inflammation as a process central to the pathogenesis of MDD writ large, available data do not support either prediction. As I discuss below, several lines of evidence suggest that anti-inflammatory agents only show an antidepressant signal in patients with elevated peripheral inflammatory biomarkers prior to treatment, and in the largest study of a cytokine antagonist conducted to date in medically healthy individuals with MDD, a strong correlation was observed between baseline inflammatory biomarkers and subsequent antidepressant response [24]. Moreover, cytokine antagonism actually appears to produce an adverse effect by blocking placebo responses in patients with lower levels of inflammation, a finding in direct contradiction to the idea that MDD might be a condition of generalized hypersensitivity to inflammatory signaling [24, 25].

I revisit this issue toward the end of the article when considering why various anti-inflammatory agents may fail as antidepressants. Now let us turn to an examination of the evidence for and against the proposition that specific anti-inflammatory modalities hold promise as antidepressants.

3 Do Anti-Inflammatory Agents Work as Antidepressants and If So When?

A number of meta-analyses have been conducted to assess the impact on depressive symptoms of agents with anti-inflammatory effects. In the current review I focus on three of these, because they are the most recent. Kohler et al. combined data from 14 trials (6,262 participants) that examined nonsteroidal anti-inflammatory drugs (NSAIDs) or cytokine antagonists in both primary MDD and for depressive symptoms in patients with medical conditions for which anti-inflammatories are primary treatment modalities [26]. They report that across these agents and conditions anti-

inflammatories reduced depressive symptoms (SMD −0.034; 95% CI −0.57 to −0.11). Rosenblat et al. combined data from 8 randomized trials (312 participants) that examined NSAIDs, omega-3 fatty acids, n-acetylcysteine, or pioglitazone as adjuncts in the treatment of depressive or mixed states in patients with bipolar disorder [27]. The overall effect size of anti-inflammatory agents on reducing depressive symptoms was −0.40 (95% CI −0.65 to −0.14). Finally, Mocking et al. followed up on several previous meta-analyses finding null effects of omega-3 fatty acids, by conducting a meta-analysis limited to patients with rigorously diagnosed MDD and reported that omega-3 fatty acids (and especially eicosapentaenoic acid [EPA]) reduced depressive symptoms with a pooled effect size of −0.398 (95% CI −0.682 to −0.114) [28].

Taken at face value these pooled effect sizes seem to suggest a level of promise for the antidepressant efficacy of inflammatory blockade that a closer examination of the data does not support. I say this because of the presence – singly and in combination – of at least three serious confounds in the extant literature. These confounds include: (1) off target (i.e., non-immune) effects of the agents; (2) the possibility that depression improved secondary to improvements in the primary immune-based disease state; and (3) irregularities/limitations in the design of the studies that disproportionately drive positive meta-analytic findings. As we shall see all three confounds are relevant to studies of NSAIDs; omega-3 fatty acids are subject to confound 1 and studies of cytokine antagonists are bedeviled by confound 2.

Prior to examining these confounds a more general point is worth highlighting, which we might call the "apples and oranges problem." There is a tendency to see inflammation as a unitary, monolithic process, but nothing could be further from the truth. Because of this, combining findings from NSAIDs and cytokine antagonists under a single anti-inflammatory rubric may hide as much as it reveals. Although both classes of agents have anti-inflammatory effects, they act at very different points in the inflammatory cascade. Cytokine antagonists specifically target cytokines, such as TNF-alpha and interleukin (IL)-12 and 23, that play primary roles in launching inflammation, whereas NSAIDs target downstream enzymes that modulate the production of arachidonic acid-derived molecules such as prostaglandins. Importantly, although prostaglandins have multiple proinflammatory properties they have also more recently been shown to play active roles in resolving inflammation. Some evidence suggests that this may explain why NSAIDs worsen outcomes in some chronic inflammatory states, such as cardiovascular disease, and why several lines of evidence suggest that they may also worsen depression, at least in some circumstances [29].

4 Off Target Effects

Because inflammatory processes are driven to a large extent by the actions of cytokines, what most of us mean when we describe MDD as an inflammatory condition is that cytokines are elevated in the disorder, and – more boldly – that elevated cytokines may be a cause of the condition. If so, then the purest test of the inflammatory hypothesis of depression would be to show that blocking proinflammatory cytokine activity treats MDD. It is for this reason that the antagonists of IL-1-beta, TNF, and IL-6 offer the most straightforward method for testing whether anti-inflammatory agents work as antidepressants. These large biologic agents have remarkable specificity of action. They block their respective cytokine targets without having other appreciable biological activities that might promote or hinder their potential antidepressant properties.

The same cannot be said for the other anti-inflammatory agents that have been tested as antidepressants and that have contributed to the effect size estimates of recent meta-analyses. Consider NSAIDs. In addition to the complication mentioned above (i.e., that they may actually have proinflammatory properties in the context of chronic inflammation), these agents have a number of depression-relevant actions not directly connected with their immune effects.

For example, celecoxib, the agent most often studied for its antidepressant properties, enhances the translocation of the glucocorticoid receptor from cytoplasm to nucleus, inhibits NA+ and K+ channels in neurons, and increases cadherin 11, an adhesion molecule that plays an important role in synaptic plasticity and that produces antidepressant- and anti-anxiety-like effects in animal models [30]. On the other hand, NSAIDs also block the CNS actions of p11. In animal models, SSRIs acutely activate cytokines in the CNS, which is necessary for p11 induction [31]. Induction of p11, in turn, is required for these agents to produce an antidepressant-like effect. Taken together, these findings suggest that NSAIDs have off target effects that might explain why they would work as antidepressants, as well as off target effects that might explain why they might not work as antidepressants.

Omega-3 fatty acids have multiple biological effects that likely contribute to their anti-inflammatory capacity, including suppression of arachidonic acid content/activity and inhibition of nuclear factor kappa-beta, as well as stimulation of g-protein receptor 120 and peroxisome proliferator-activated receptor (PPAR)-gamma [32, 33]. But like NSAIDs, omega-3 fatty acids have a number of off target effects that might contribute to an antidepressant effect. In addition to wide ranging effects on membrane stability and function, both EPA and DHA have been shown to promote neurogenesis independently of effects on inflammation, which – in animal models at least – appears to be an important prerequisite for inducing antidepressant-like effects [34].

5 Antidepressant Effects Secondary to Improvement of the Underlying Medical Disease

A second potential confound in meta-analyses of the antidepressant effect of anti-inflammatory agents derives from the fact that many of the included studies examined populations with medical diseases that are likely to benefit directly from anti-inflammatory therapies. For example, three of the four cytokine antagonist studies included in the Kohler et al. meta-analysis examined patients with psoriasis, and five of the ten NSAID studies (including all that evaluated NSAIDs as monotherapy for depression) examined patients with active and symptomatic osteoarthritis (OA) [26].

The obvious challenge posed by the inclusion of these studies is that anti-inflammatories may have antidepressant properties in these illnesses primarily because they reduce primary disease symptoms that are contributing to the depression in the first place. Indeed, both psoriasis and osteoarthritis are associated with high levels of depression, raising the possibility that the effective treatment of these disease states might reduce depression in and of itself. If so, then the antidepressant effects of anti-inflammatories should be associated with their ability to improve underlying disease state symptoms. In fact, this was the case for both the cytokine antagonist and NSAID studies included in the Kohler et al. meta-analysis that examined patients with psoriasis or osteoarthritis. In the five included studies that compared ibuprofen, naproxen, celecoxib with placebo in patients with osteoarthritis improvement in OA symptoms was strongly associated with improvements in depressive symptoms. [35] A similar picture emerges from studies examining the impact of cytokine antagonists on depressive symptoms in patients with psoriasis. While a large study that compared the TNF antagonist etanercept with placebo found that improvements in depression did not correlate with improvements in psoriasis symptoms (although improvements in fatigue did correlate with improvements in psoriasis) [36], two subsequent studies found medium to large effect–size correlations between improvements in depression and psoriasis in response to treatment with the TNF antagonist adalimumab ($r = 0.50$, $p < 0.0001$) and the IL-12/IL-23 antagonist ustekinumab ($r = 0.32$, $p < 0.0001$) [37, 38].

6 Limitations in Study Design and Irregularities in the Presentation of Findings

We have highlighted the fact that studies examining the antidepressant effects of anti-inflammatories in patients with psoriasis or OA suffer from confounds. These studies in medically ill patients suffer from another limitation, which is that they did not specifically enroll participants with clinically significant levels of depression. In the Tyring et al. study of etanercept in psoriasis only 15% of 618 participants entered the study with depressive symptoms of severity sufficient to qualify for

entry into most antidepressant trials [36]. Similar low levels of depressive symptom severity also characterized the Langley et al. study of ustekinumab in 1,230 patients with psoriasis (11% with moderate or greater severity depressive symptoms) [38]. In the final cytokine antagonist study in psoriasis approximately 33% of participants qualified for having depression based on a Zung score $\geq$50 [37]. In the 5 trials comparing celecoxib, naproxen, ibuprofen, and placebo in patients with OA, baseline scores were even lower (average score of 3 on the 9-item Patient Health Questionnaire, with moderate depression starting at a score of 15) [35].

The same issue plagues the largest study of healthy individuals included in the Kohler et al. meta-analysis ($N = 2233$) [39]. This trial examined the effects of celecoxib vs. naproxen vs. placebo on depressive symptoms cognitively normal adults over the age of 70. Despite the large sample size, however, only 1/5 of the study subjects had "significant depression" defined by cut-off score of >5 on the Geriatric Depression Scale. No effect of NSAID treatment was seen on depression scores in the population as a whole, or in participants who entered the trial with elevated depressive symptom scores.

These low levels of depressive symptoms have the potential to introduce a "floor effect" that might well obscure efficacy had these studies been conducted in participants with clinically relevant depression. Convergent support for this possibility comes from the literature examining omega-3 fatty acids. A recent meta-analysis that only included participants with rigorously defined MDD found a larger effect size for omega-3s than did earlier meta-analyses that included participants with lesser degrees of depression did not [28]. And in a negative meta-analysis conducted by Bloch and Hannestad, increasing baseline depressive symptom severity was associated with a larger effect size difference between omega 3s and placebo [40].

A recent meta-analysis of anti-inflammatory agents in patients with bipolar disorder included two studies of NSAIDs. One study examined their efficacy as augmenting agents in patients currently in a depressed or mixed state and found no effect [41]. The other study examined the addition of aspirin to euthymic patients with bipolar disorder and – not surprisingly given the patients' baseline status – found no effect [42]. On the other hand, the Kohler et al. meta-analysis found evidence for an anti-inflammatory effect of NSAIDs based on the inclusion of four trials that examined the impact of augmenting standard antidepressants with the selective cyclooxygenase (COX) 2 inhibitor celecoxib in medically healthy individuals with diagnosed major depression.

But, as with the literature more generally, issues with these studies suggest caution in our interpretation of their findings. Muller et al. conducted a well-designed and described study that has received significant attention since its publication in 2006 [43]. In this study 40 individuals with DSM-IV diagnosed major depression were randomized on a 1-to-1 basis to 6 weeks of reboxetine plus celecoxib or 6 weeks of reboxetine plus placebo. Although drop-out rates were very high (i.e., 10 in the celecoxib group and 9 in the placebo group), at the end of the trial a last-observation-carried-forward methodology found a significantly larger

improvement in depressive symptoms in the group randomized to adjunctive celecoxib than to adjunctive placebo (effect size calculated as $d = 0.58$).

Given the small sample size and high drop-out rate, results from the Muller et al. study should certainly be considered suggestive and intriguing rather than definitive. Deeper difficulties plague the remaining three studies of celecoxib augmentation of SSRIs. Two of these studies were conducted by the same research group, based at the Tehran University of Medical Sciences [44, 45], and a third small trial was published by another group in Iran (Moshiri et al.) [26]. Both studies from the Tehran University of Medicine group show strikingly large effect size advantages for celecoxib vs. placebo augmentation (calculated by us as $d = 1.09$ for Akhondzadeh et al. and reported as $d = 0.95$ for Abbasi et al.).

Intriguingly, the absolute difference in change scores between randomized groups in these studies was quite modest – approximately 3 points on the 17-item Hamilton Depression Rating Scale. To show statistical significance for this type of difference (which is typically what antidepressants deliver), pharmaceutical concerns in the west need to enroll at least 100 participants per randomized arm, consistent with the fact that effect sizes for antidepressant trials are typically a third of those observed in the two Iranian-based trials of celecoxib augmentation. So how did the two celecoxib augmentation studies achieve such large effect sizes and concomitant statistical significance with such small populations and modest between-group differences in mean symptom change?

The answer lies in the fact that the Iranian study samples showed little variation in outcomes (i.e., the standard deviations for change scores in both study arms are very small). A similar pattern of small variations in outcome and very large effect sizes has been reported by this group for a number of non-traditional interventions in psychiatric conditions (i.e., effect size of 1.76 for crocus sativus [saffron] as an antidepressant) [46], strongly suggesting that the relevant subject populations are qualitatively different from those recruited in other cultural milieus. Although the third study of adjunctive celecoxib reports more modest statistical differences between active treatment and placebo as a result of using more rigorous non-parametric statistics appropriate to the small sample size, the absolute differences in change score between celecoxib and placebo were similar to those observed in the Tehran University studies. Taken together, these considerations suggest that caution may be in order regarding any expectation that NSAID augmentation will show similarly large effects in other sociocultural settings.

7 What Can We Learn from Cytokine Antagonism in Medically Healthy Adults with MDD?

It is an interesting paradox that in a field filled with studies there is, to my knowledge, only one randomized, double-blind, placebo-controlled study in the world's literature to date that utilizes an anti-inflammatory agent with no "off-

target" effects (infliximab) in patients with rigorously defined major depression [24]. Because we conducted the study I am especially aware of its limitations and weaknesses. Nevertheless, because it is the only study of its type, I suggest that it provides the most direct insights currently available into the question of whether anti-inflammatory activity, per se (and cytokine blockade in particular), will emerge as an "all-purpose" antidepressant mechanism.

This study randomized 60 medically healthy adults with treatment-resistant major depression (defined as a score ≥ 2 using the Massachusetts General Hospital Staging method) to either three infusions of the TNF-alpha antagonist infliximab (5 mg/kg) vs. three infusions of salt water placebo. Infusions were delivered at baseline, study week 2, and study week 6 and clinician- and self-report-based assessments of depressive symptoms and related constructs were obtained at baseline (i.e., pre-treatment) and at study weeks 1, 2, 3, 4, 6, 8, 10, and 12. Enrolled subjects were either off antidepressants or on a stable antidepressant regimen for at least 4 weeks prior to study entry without appreciable clinical response. Subjects who entered on an antidepressant regimen were required to maintain this regimen throughout the study period. Ninety percent of the randomized sample completed the 12-week study.

The results from the study were unequivocal. The groups were as close to each other in outcome as could be expected by chance (i.e., $p = 0.92$), and – in fact – placebo outperformed infliximab on a numeric basis. These findings do not auger well for the hypothesis that cytokine blockade holds promise as an "all purpose" antidepressant modality, with the caveat that placebo rates were strikingly high (i.e., around 50%) which might have obscured real – but small – antidepressant effects of the infliximab.

Interestingly, however, the similar responses to placebo and infliximab hid a complexity that I believe provides an important key to understanding not just the antidepressant potential of anti-inflammatory agents, but the relationship between inflammation and MDD more generally. We entered the study predicting that increased measures of peripheral inflammation prior to the receipt of a study intervention would be associated with an improved response to infliximab, but not placebo. This hypothesis turned out to be truer than we would have guessed based on what we understood about the association between inflammation and depression at the time we designed the study. As expected, a linear relationship was observed between increasing plasma concentrations of high-sensitivity c-reactive protein (hs-CRP) and antidepressant response to infliximab and TNF. What we didn't expect was that this relationship would show a true dose–response pattern, meaning that depressed participants with low levels of baseline peripheral inflammation did *worse* on infliximab than placebo. Because we expected a null relationship between placebo administration, inflammation, and antidepressant responses, we also did not predict that increasing peripheral inflammation would be associated with *reduced* placebo responses, but that is what we found.

The "sweet spot" for infliximab effectiveness was an hs-CRP plasma concentration of 5 mg/L. Participants with inflammatory activity above this level did better with infliximab than placebo, with a medium effect size of 0.41, which is in line

with the efficacy of antidepressants against placebo in most studies. On the other hand, participants with hs-CRP below 5 mg/L did better on placebo than infliximab (effect size 0.82). Importantly, in participants with hs-CRP levels about 5 mg/L the response to infliximab was not the result of only impacting "sickness symptoms" such as fatigue, but resulted from a reduction in the core major depressive disorder (MDD) symptoms of depressed mood and anhedonia, and from other symptoms often considered "emotional" as opposed to "somatic," including suicidal ideation and psychic anxiety.

8 Facing the Etiologic and Treatment Implications of the U-Shaped Curve

Results from our infliximab study await replication. Pending this, it is striking that an exactly similar pattern of findings was observed in a study that examined EPA vs. docosahexaenoic acid (DHA) vs. placebo as monotherapy in MDD [25]. Neither of the omega-3 fatty acids showed any evidence of superiority over placebo in the group as a whole. But EPA showed a large effect size advantage over both placebo and DHA in participants with increases in any of a number of inflammatory biomarkers at baseline. Conversely, depressed participants with low levels of inflammatory markers actually did worse on omega-3s than on placebo, exactly as we observed with infliximab.

These findings present us with a conundrum. We know from studies with both acute inflammatory stimulators (i.e., LPS) and chronic stimulators (i.e., IFN-alpha) that individuals vary in their sensitivity to inflammation. For any given "dose" of inflammatory exposure some individuals get far more depressed than others. This suggests that even low levels of inflammation should be depressogenic in vulnerable individuals, and that because of this they might well benefit from an anti-inflammatory intervention. But, as we've seen, treatment studies do not support this, and in fact suggest the opposite: that blocking inflammatory signaling in depressed individuals with low levels of inflammation is actually counter-productive.

In beginning to resolve this paradox two points are important to consider. First, studies of the behavioral effects of acute cytokine stimulation are universally conducted in participants without clinical depression. Second, the doses of IFN-alpha used in treatment studies are so high that all individuals are being exposed to levels of inflammatory stimulation that far exceed anything relevant for individuals with MDD and low levels of inflammation. It may be that such high chronic cytokine exposure distorts relationships between the immune system and brain/neuroendocrine pathways that pertain at lower levels of inflammatory signaling.

That the relationship between inflammation and depression might be complex (i.e., non-linear or "u-shaped") has been suggested by studies in the literature that

have not gotten the attention they deserve. For example, significant evidence from animal studies that at lower concentrations inflammatory cytokines in the CNS play a pivotal role in learning and memory and other processes in the brain that maintain neuronal integrity including synaptic plasticity [47]. In addition to the importance of the amount of inflammation present at any given time, it may well be that inflammatory activity has different effects on depression depending on its timing relative to initiating environmental causes. For example, blocking CNS microglial activation at the onset of a chronic unpredictable stressor (CUS) abrogated the later development of depressive-like symptoms in a rodent model, consistent with the likely role of inflammation as a transducer of environmental stress into behavioral pathology [48]. But paradoxically, once mice had been exposed to the CUS, anti-inflammatory interventions worsened their depressive and anxiety-like behavior, whereas treatment with several inflammatory stimulators (including LPS) actually reversed the already-existent depressive-like behavior, and did so in concert with stimulation of hippocampal microglial proliferation.

Is it possible that acute inflammatory stimulation may produce depression in humans who are not depressed, but have antidepressant properties in patients – who like the mice already exposed to the chronic stressor – have endured long-term activation of stress pathways in the brain and body? Although this sounds far-fetched, at least one study in humans suggests this idea may have some merit. In a small open trial conducted in the 1990s, Bauer et al. administered LPS to seven melancholically depressed adults and monitored sleep using polysomnography for two nights prior to, and two nights following the LPS administration [49]. LPS increased plasma concentrations of TNF-alpha and IL-6, suppressed REM sleep, and produced a significant reduction in depressive symptoms the following day. The more IL-6 increased in response to LPS, the more depressive symptoms decreased the following day. Upon recovery sleep the next night, 5 of the 7 subjects relapsed, but 2 continued to show improved depression scores. The limitations of this type of small, open trial are obvious, but the results are nonetheless intriguing, and when coupled with animal data showing that inflammatory cytokines play important roles in healthy brain functioning when not chronically elevated.

9 Conclusions

Although much work remains to be done, data collected to date suggests that the role of inflammatory cytokines as pathogenic agents in major depression is likely limited to a subset of patients with evidence of inflammatory hyperactivity. Fortunately, increasing data suggest that easily obtainable measures of inflammation, such as hs-CRP, hold promise as markers for the sub-group of depressed individuals most likely to benefit from anti-inflammatory treatment strategies. But the converging lines of evidence suggesting that cytokines may have positive effects at lower concentrations or as acute stimuli in the context of severe depression/chronic stress highlight the need for restraint in our desire to apply a “cookie-cutter”

approach to the use of anti-inflammatories in the treatment of depression more generally.

Finally, for all the reasons I've discussed, anti-inflammatory agents are unlikely to be antidepressants as the term is typically conceived. But in this regard they may be no different than other agents currently approved for the treatment of MDD. Recent mathematical modeling suggests that behind the modest differences in mean change scores typically observed between antidepressants and placebo hides a more complex truth. Based on a large subject sample, John Crystal's group at Yale has shown that approximately 75% of patients who receive antidepressants obtain significant short-term clinical benefit [50]. However, 25% of patients actually do much worse on antidepressants than on placebo. This result, and others like it [51], strongly resembles our findings with infliximab in treatment-resistant depression and the findings of Rapaport et al. with omega-3 fatty acids. The only difference may be that in the case of anti-inflammatory interventions, we have biomarkers that make who does and doesn't respond seem a little less mysterious than is the case with classical antidepressants.

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Are Non-steroidal Anti-Inflammatory Drugs Clinically Suitable for the Treatment of Symptoms in Depression-Associated Inflammation?

Bernhard T. Baune

Abstract The aetiology and pathophysiology of depression have long been associated with inflammation, at least in a proportion of patients. Altered cytokine activity in the periphery and in the brain has brought support to a concept of depression-associated inflammation. However, these immunological changes – and inflammation in particular – in depression have only been recently targeted for treatment. Non-steroidal anti-inflammatory drugs (NSAIDs) have been proposed to be of clinical use in the treatment of depression either as monotherapy or as adjuncts in combination with antidepressants. Specifically, selective cyclooxygenase (COX)-2 and non-selective COX inhibitor NSAIDs as adjuncts or monotherapy have been trialled clinically. A limited body of clinical research has been conducted with mixed results so far. Although meta-analyses appear to support the use of NSAIDs in acute depression, the overall effect is mainly biased by the effects of celecoxib for which the best evidence exists to date. Efficacy data of non-selective COX inhibitor NSAIDs on depressive symptoms is limited and out of six studies, only a retrospective analysis shows positive results for non-selective COX inhibitor. Clinical data on aspirin, an irreversible inhibitor of both COX-1 and COX-2, are mainly experimental and hypothetical at this stage, but may be promising in depressed patients with concomitant inflammatory conditions. The main problematic factor is that current evidence rests on trials in acute depression. Because of the dynamic nature of depression, it is important exploring if NSAIDs and other anti-inflammatory treatments may have a preventive role in early stages of depression and for relapse prevention. The possible impact of anti-inflammatory treatments on immune changes in different phases of depression warrants caution

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for a wide and preventive use of anti-inflammatory agents in depression-associated inflammation.

Keywords Depression-associated inflammations • Non-steroidal anti-inflammatory drugs • Phases of depression • Prevention

Contents

1 Introduction: Symptoms and Mechanisms of Depression-Associated Inflammation

Novel treatment strategies for depression are urgently needed. Recent global data suggests unipolar depression currently ranks 11th for disability adjusted life years, a 37% increase since 1990 [1]. The burden is expected to continue to grow into the twenty-first century [1, 2]. Hence, this is an unprecedented burden of depressive illness requiring increased effort to find novel therapeutic agents for treatment [3]. Additionally, more than 50% of patients on antidepressants will not achieve remission following initial treatment [4], and nearly one-third will not achieve remission following several treatment steps [5, 6].

While the pathophysiology of depression still requires understanding of key mechanisms that are translatable into treatment approaches, the role of inflammation and immune activation more generally in depressive symptoms has long been discussed and recently gained momentum as a potential treatment target. Growing evidence suggests that pro-inflammatory cytokines play a major role in the pathophysiology of depression. A number of studies, both experimental and meta-analytic analyses, suggest an increased expression of pro-inflammatory cytokines, TNF-α, IL-1β and IL-6, in the brain of patients with major depressive disorder (MDD) [7–10]. A seminal meta-analysis [7] of 24 studies found significantly higher concentrations of the pro-inflammatory cytokines, tumour necrosis factor (TNF)-α

and interleukin (IL)-6, in depressed subjects compared with control subjects. An updated meta-analysis [11] of IL-6, C-reactive protein (CRP) and TNF-α found higher levels of IL-6 and CRP in depressed patients versus controls (29 studies for IL-6 and 20 for CRP). These studies strengthen the clinical evidence that symptoms of depression can be accompanied by an activation of the inflammatory response system [7].

Medical illnesses can induce a variety of biological and behavioural responses, including fever, anorexia, weight loss, fatigue, sleep disturbances, motor retardation, dysphoria, anhedonia, impaired cognition and depressed mood, which are all seen in major depression. Administration of pro-inflammatory cytokines can induce such symptoms in animals [12] and humans [13–15]. These symptoms are often referred to as sickness behaviour. The biological pathways by which cytokines may mediate depression are poorly understood, though a number of mechanisms have been proposed:

1. The 'pro-inflammatory cytokines' affect serotonin (5-HT) metabolism by reducing tryptophan (TRP) levels. Cytokines appear to activate indoleamine-2-3-dioxygenase (IDO), an enzyme which metabolizes TRP, thereby reducing serotonin levels. Furthermore, inflammatory cytokines, such as IL-1β, may reduce extracellular 5-HT levels, via activation of the serotonin transporter mechanisms.
2. Pro-inflammatory cytokines have a potent direct effect on the hypothalamic–pituitary–adrenal (HPA) axis. Cytokines, including IL-1, IL-6, TNF-α and IFN-α, have been shown to increase inflammatory responses by disrupting the function of glucocorticoid receptors (GRs).

Infection and tissue damage can lead to increased local cytokine production both within and outside of the CNS. Cytokines are large, hydrophilic molecules, which do not easily cross the blood brain barrier under normal conditions. Peripheral cytokines may, however, communicate with the cerebrum through various pathways. These include communication with the CNS through the afferent sensory fibres of the vagus nerve. Other pathways of communication can occur through passive diffusion at areas where the blood brain barrier is deficient or by active transport of cytokines stimulated by the central noradrenergic system. Peripheral cytokines may also activate neural afferents, leading to synthesis of IL-6 within the brain by microglia and endothelial cells [16–18].

In the CNS, cytokines may exert their effects by activating the HPA axis. Pro-inflammatory cytokines induce gene expression and synthesis of corticotrophin releasing factor (CRF), which stimulates adrenocorticotropic hormone (ACTH) release and causes glucocorticoid secretion [19]. An activated HPA axis may lead to a further rise in pro-inflammatory cytokines, through a complex positive feedback loop. Stress can lead to increased cytokine levels and an induction of catecholamines via an activation of the HPA axis, which may further increase pro-inflammatory cytokines [18]. Cytokines may also directly affect higher cognitive and emotional functions [20], possibly leading to depression and the associated cognitive dysfunction.

Inflammation and cytokines in particular as carriers of inflammation have been shown to exert a variety of neurobiological effects relevant to depression. Specifically, cytokines such as TNF-α, IL-1β, IL-6 and interferon (IFN)-γ impact key neurobiological processes such as neuroplasticity, neurotransmission, oxidative stress and neuroendocrinological functions that are considered to be central to the development of depression [20–24]. Pro-inflammatory cytokines impair hippocampal (HC) neuroplasticity (e.g. neurogenesis, synaptic plasticity and long-term potentiation (LTP)), induce glucocorticoid insensitivity of the HPA axis, increase oxidative stress in the HC, reduce serotonin (5-HT) levels and create neurotoxic serotoninergic metabolites (i.e. 3-hydroxykynurenine (3-HK) and quinolinic acid (QA) [21, 24–27]. Astrocytes and microglia also key components of the innate immune system also play a role in the pathophysiology of depression [22]. Microglia that are a major producer of pro-inflammatory cytokines and are important regulators of HC neuroplasticity, oxidative stress and QA have increased activity in depression [28]. Microglia can cause detrimental processes when activated, while producing beneficial processes when quiescent. A recent study [28] used positron emission tomography to better understand neuroinflammation in depression. The binding activity of the TPSO in microglia is indicative of microglia activity and related inflammatory processes in the brain. Investigators found increased binding in all brain regions, especially the prefrontal cortex, anterior cingulate cortex and insula in depressed subjects. Moreover, astrocytes that have shown a prominent role in releasing neurotrophic and anti-oxidant factors have also been found to be activated in depression [29]. A range of clinical and pre-clinical studies indicate astrocytic abnormality in depression, leading to detrimental processes in the brain [30].

2 Pharmacological Approaches to Depression-Associated Inflammation

2.1 Non-steroidal Anti-Inflammatory Treatments in Depression

Given the clinical and mechanistic relationship between inflammation and depression, both selective cyclooxygenase (COX)-2 and non-selective COX inhibitor non-steroidal anti-inflammatory drugs (NSAIDs) have been investigated as possible adjuncts in the treatment of depression with antidepressants [31–44]. The results are mixed based on various study designs ranging from retrospective cohort studies [32–34, 36, 41], randomized-controlled trials (RCTs) [34, 38–40] to nested case-control studies [41]. While some studies have found positive antidepressant effects [31, 38, 40, 41], others have found no effect [32, 34, 43] and yet others have found detrimental effects suggesting NSAIDs may reduce the antidepressant effect of selective serotonin reuptake inhibitors (SSRIs) [36, 44]. These mixed results may

be due to various reasons such as differing antidepressant classes and doses, the use of varying selective COX-2 and/or non-selective COX inhibitor NSAIDs, study design, age of study population, as well as the consideration of populations with varying degrees of depressive symptomatology and presence of comorbid medication conditions.

NSAIDs are classically thought to have a beneficial effect on the brain in depression [45], thought to be due to their ability to reduce the central and peripheral pro-inflammatory state commonly seen in depression [22]. COX-1 is a major player in mediating pro-inflammatory microglial activation [46], hence blocking this enzyme should be beneficial. Moreover, recent evidence indicates that depression is accompanied by increased COX-2 activity [47, 48], hence postulating a role of COX-2 in depression pathophysiology. Galecki et al. [48] found mRNA expression of genes encoding for COX-2 were significantly increased in the peripheral blood cells of recurrent depressive disorder patients ($n = 181$) vs. controls ($n = 149$).

The differing efficacy of selective COX-2 vs. non-selective COX inhibitor NSAIDs raises important questions about their pharmacological actions. Our understanding of the physiological and pathophysiological effects of COX-1 and 2 is still limited. For example, COX-1 is predominantly pro-inflammatory, mediating pro-inflammatory microglial activation [46], and is considered detrimental to the brain [49], whereas COX-2 can exert both beneficial and detrimental effects on the brain (see, for review, [50]). A recent meta-analysis of RCTs suggests that celecoxib, a selective COX-2 inhibitor NSAID, has a therapeutic effect when used adjunctively with antidepressants [51]. However, mechanistic evidence suggests that selective COX-2 inhibitor NSAIDs may actually increase neuroinflammation, Th1 immune responses and glial cell activation [46, 49, 52]. Various mechanistic explanations for the inconsistent effects of NSAIDs in the clinical treatment of depression need to be considered. Some NSAIDs may have more efficacy than others based on COX enzyme effects; however, this is still subject to debate [46, 49, 52]. Evidence from rodent studies indicates that by-products of COX-2 metabolism (e.g. prostaglandin D2 and docohexanoic acid) exert anti-inflammatory effects [49]. Given this, aspirin is thought to be a more desirable compound given it preferentially targets COX-1 over COX-2 [50]. COX-1 may play a more significant role in neuroinflammation in depression-like states; however, this is poorly understood [49, 50, 52]. Some evidence indicates that NSAIDs may have a minimal and potentially detrimental effect on depressive symptoms by attenuating the effects of SSRIs [44]. A rodent study by Warner-Schmidt et al. [44] shows that SSRIs increase TNF-α, IFN-γ and p11 levels in the frontal cortex. The NSAID, ibuprofen, reduced these levels and attenuated the antidepressant-like actions of SSRIs, but not of tricyclic or monoamine oxidase inhibitors. In a rodent study, it was attempted to differentiate the effects of COX-1 and COX-2 enzymes on systemic immune cell migration [53]. This study found that the inhibition of COX-1 activity reduces leukocyte recruitment into the inflamed CNS (via intracerebroventricular lipopolysaccharide), whereas selective COX-2 inhibition increases this recruitment [53], suggesting differential chemotactic effects of these enzymes on the brain. These

mechanistic findings may assist to explain the variety of clinical study results found in human trials on the therapeutic effects of NSAIDs in depression and they suggest that the design of clinical trials requires the consideration of different pharmacological properties. In addition, effects of NASAIDs on depressive symptoms may depend upon the presence of an inflammatory state that has been shown repeatedly in patients with comorbidities such as osteoarthritis or psoriasis and depressive symptoms [51]. Unfortunately, clinical trials of NSAIDs in depression have not stratified patients according to the presence of inflammation yet.

2.2 *Is There a Role for Aspirin in the Treatment of Depression-Associated Inflammation?*

Aspirin is an NSAID, and an irreversible inhibitor of both COX-1 and COX-2. It is more potent in its inhibition of COX-1 than COX-2, and targeting COX-2 alone may be a less viable therapeutic approach in neuropsychiatric disorders such as depression. While some clinical evidence indicates beneficial effects for aspirin in mood disorders through a shortened onset of action of antidepressants [54], negative results come from epidemiological analyses of 5,556 older men, which showed no association between current aspirin use and depression [32]. In an intervention study of 70 patients with depression, administration of aspirin together with fluoxetine conferred a greater reduction of oxidative stress markers compared with fluoxetine monotherapy [55]. More generally it is currently investigated if low dose aspirin has the potential to extend a healthy process of aging and disability free life among the elderly in the "*ASPirin in Reducing Events in the Elderly (ASPREE)*" study [56]. As ASPREE will examine whether the potential primary prevention benefits of low dose aspirin outweigh the risks in older healthy individuals, its secondary endpoints include all-cause and cause specific mortality, fatal and non-fatal cardiovascular events, fatal and non-fatal cancer (excluding non-melanoma skin cancer), dementia, mild cognitive impairment, depression, physical disability and clinically significant bleeding. This study will be providing data and insight into the preventive effects of low dose aspirin on depression development with and without comorbidities characterized by inflammation (e.g. osteoarthritis, cardiovascular diseases and cancer). At this stage of the literature, it is too early to conclude any replicated clinical effects of aspirin in depression whether with or without concomitant inflammatory state, hence, the current state of knowledge for aspirin in depression is mainly experimental and hypothetical.

2.3 *Anti-Inflammatory Interventions Using Targeted Antagonists*

A range of biological antagonists are available such as Infliximab, a chimeric bivalent IgG1 monoclonal antibody composed of a human constant region and murine variable regions, adalimumab – a humanized bivalent mouse IgG1 monoclonal antibody and etanercept – a fusion protein comprised of human IgG fused to a dimer of the extracellular regions of TNF-α-R-2. Additionally anti-TNF therapy is being considered as an option in improving postoperative cognitive dysfunction [57]. Research into the effects of centrally and peripherally administered TNF-α blockade has shown clinical efficacy on cognition and depressive symptoms and has improved our understanding of cytokine actions in the CNS [58–60]. In addition, a recently published trial in an animal model under peripheral LPS stimulation showed that centrally administered etanercept reduced anxiety-like behaviours, but not spatial memory and was associated with a decrease in hippocampal microglia numbers being suggestive that etanercept recovers anxiety-like behaviour possibly mediated by a reduction of TNF-α related central inflammation [61].

However, there are two problems with this approach. First, the BBB prevents a deep penetration of the monoclonal antibody into the CNS tissue and second, while basal levels of TNF-α are still required for normal functioning, animal models have shown that the complete lack of TNF-α due to genetic modification results in cognitive impairment [62]. This is possibly due to the influence that TNF-α exerts on NGF and BDNF. An imbalance in TNF-α causes subsequent deregulation in these neurotrophins ultimately leading to morphological changes in the hippocampus such as decreased arborization of pyramidal neurons [63]. Additionally, the role TNF-α plays in long-term potentiation (LTP) and depression (LTD) formation by up-regulation of AMPA receptors [64] and endocytosis of GABA receptors [65] is crucial in the development of synaptic neuroplasticity related memory and learning [20]. Therefore, though complete blockage may prove to be initially beneficial, long-term negative effects may manifest and make further research into these effects necessary. The complex signaling pathways of TNF-α and its receptors and the duality of its function in being both neuroprotective and neurodegenerative make for a compelling argument against the validity and long-term benefits of anti-TNF-α therapies. TNF-α may both exacerbate and attenuate cognitive dysfunction depending on the physiological context [66, 67]. Clearer perspectives into TNF-α signaling and pharmacological interventions that can target specific apoptotic factors in the TNF-α receptor-associated pathways, rather than complete blockage of TNF-α or of its receptors would make for more effective therapeutics in treatment of neurological disorders in which TNF-α is an active participant.

Investigations of alternative pathways to inhibit neuroinflammation show promising results. In a study examining the effect of ubiquitin-specific processing protease 8 (USP8) in luteolin-treated microglia, it was found that luteolin inhibits microglial inflammation by enhancing USP8 protein production and USP8 might represent a novel mechanism for the treatment of neuroinflammation and

neurodegeneration [68]. In addition, it has been shown that blocking the kinase activity of RIP1, a key druggable target in the necroptosis pathway, by necrostatins inhibits the activation of necroptosis and allows cell survival and proliferation in the presence of death receptor ligands [69]. Hence, targeting RIP1 kinase may provide therapeutic benefits for the treatment of human diseases characterized by necrosis and inflammation. Although still experimental and hypothetical, a robust RIP3-dependent necroptosis signaling pathway in TLR-activated microglia upon caspase blockade has been reported that suggests that TLR signaling and programmed cell death pathways are closely linked in microglia, which could contribute to neuropathology and neuroinflammation [70]. A role of RIP3-dependent necroptosis has also been suggested for TNF-alpha-induced toxicity of hippocampal neurons. Specifically, it has been shown that TNF-alpha promotes CYLD-RIP1-RIP3-MLKL-mediated necroptosis of hippocampal neurons largely bypassing ROS accumulation and calcium influx [71]. Taken together, these RIP1/RIP3 dependent pathways in TLR-activated microglia and TNF-alpha-induced toxicity may present future targets for intervention provided future research extends these experimental findings.

3 Phases of Depression and Dynamic Immune Changes: A Concept for Targeted Anti-Inflammatory Interventions

Given the highly dynamic nature of clinical depression with different phases ranging from pre-symptomatic, first episode, recurrent episode to a high proportion of chronicity of the disease, it is important to consider anti-inflammatory treatment effects according to the clinical phase of depression. Different phases of depression can be distinguished. A pre-clinical phase of depression is characterized by sub-threshold symptoms and risk factors for the development of depression. In this phase, a proportion of people may not go on to develop a depressive episode whereas some patients may develop a first or recurrent major depressive episode. When symptomatology is not self-limited and endures or reaches higher severity, an onset of a clinical phase of depression occurs during which the depressive symptoms may be mild, moderate or severe and may include melancholic, atypical, psychotic and non-melancholic symptomatology. This clinical phase can be a first episode for an individual and interestingly, studies suggest that 60–90% of adolescents with depressive episode show remission within 1 year [72, 73]. However, 50–70% of remitted patients suffer $\geq$1 subsequent depressive episodes within 5 years [74]. Importantly, only a few adults experience a full symptomatic and functional remission between depressive episodes with a high risk of long-term loss of function [75, 76].

In addition to the variation in clinical symptomatology according to the phase of depression, the biology of inflammation and related immune alterations may also depend on the stage of illness. Hence, our group has recently suggested a staging

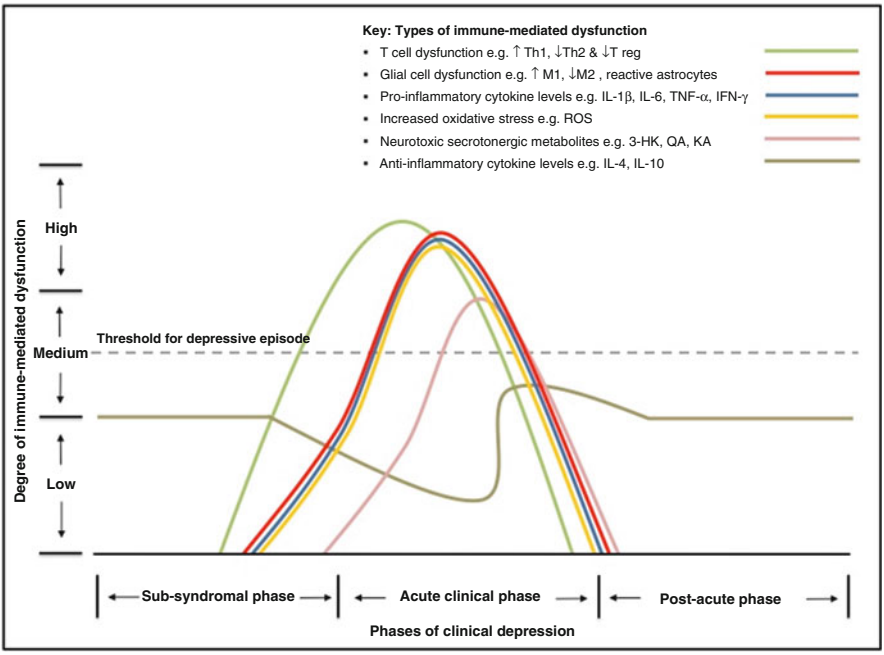

Fig. 1 A phase-specific neuroimmune model of clinical depression with remission. This figure represents an acute clinical depressive episode with full remission in the context of the 3 phases of the phase-specific neuroimmune model of clinical depression – sub-syndromal, acute clinical and post-acute phases. The *x*-axis shows the relevant phases; the *y*-axis shows the level of immune-mediated dysfunction which can occur. The *coloured lines* represent the various types of immune-mediated dysfunction. The *grey dashed line* shows the immune dysfunction threshold line whereby a clinically significant depressive episode is diagnosable. *Abbreviations*: *IL* interleukin, *TNF* tumour necrosis factor, *IFN* interferon, *BDNF* brain-derived neurotrophic factor, *LTP* long-term potentiation, *ROS* reactive oxygen species, *3-HK* 3-hydroxykynurenine, *QA* quinolinic acid, *KA* kynurenic acid, *Th* T helper, *T reg* T regulatory cell

model of inflammation in depression according to three phases [77]: *sub-syndromal*, *acute clinical* and *post-acute*. The model postulates that changes of the innate and the adaptive immune system in depression vary according to the phase of the illness as outlined in Figs. 1 and 2. The phases are represented by various interrelated neuroimmune, neuroplasticity and neuroprotection changes. Taken together, these stages represent a potential range of clinical scenarios relevant to depression and to anti-inflammatory treatment. While at this stage of the research, studies on anti-inflammation effects in depression-related inflammation have investigated the effects of anti-inflammatories on acute phases of depression. Hence, future studies need to investigate whether anti-inflammatory treatments may have clinical efficacy at pre-symptomatic stages to prevent full-onset of the disease and whether relapse prevention can be achieved using anti-inflammatory treatments (Fig. 3).

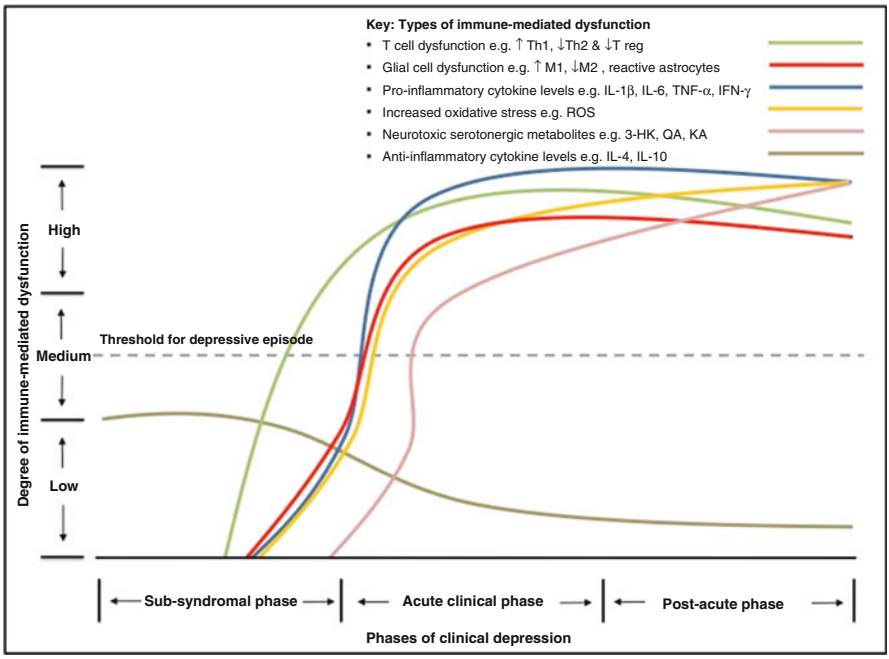

Fig. 2 A phase-specific neuroimmune model of clinical depression: Chronic major depressive episode with progressive depressive features and cognitive dysfunction. This figure represents a chronic major depressive episode with progressive depressive features and cognitive dysfunction. The *x*-axis shows the relevant phases (sub-syndromal, acute clinical and post-acute); the *y*-axis shows the level of immune-mediated dysfunction which can occur. The *coloured lines* represent the various types of immune-mediated dysfunction. The *grey dashed line* shows the immune dysfunction threshold line whereby a clinically significant depressive episode is diagnosable. *Abbreviations*: *IL* interleukin, *TNF* tumour necrosis factor, *IFN* interferon, *BDNF* brain-derived neurotrophic factor, *LTP* long-term potentiation, *ROS* reactive oxygen species, *3-HK* 3-hydroxykynurenine, *QA* quinolinic acid, *KA* kynurenic acid, *Th* T helper, *T reg* T regulatory cell

4 Caution Warranted Using Anti-Inflammatory Agents in Depression

It is important to note that the short- and particularly the long-term use of NSAIDs either for acute or preventive treatment of depression-associated inflammation carry a variety of caveats that need careful consideration. This is not to undermine the potential clinical utility of anti-inflammatory agents in depression, but to be cautious that the apparent simplicity of the idea that anti-inflammatory agents improve depressive symptoms in depression-associated inflammation does not lead to an uncritical practice. These factors include pharmacological properties of anti-inflammatory agents as well as dynamic clinical and immunological changes that can occur during various phases of clinical depression.

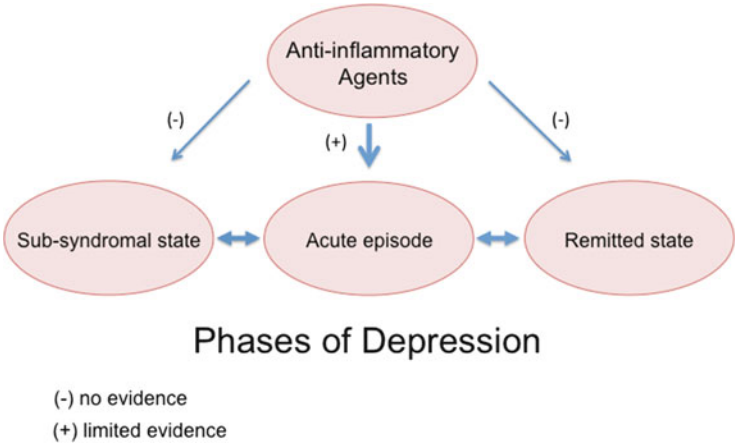

Fig. 3 Level of evidence for treatment of phases of depression with anti-inflammatory agents

4.1 Complex Pharmacology of Anti-Inflammatory Agents and Dynamic Course of Depression

Key issues based on the pharmacology of anti-inflammatory agents and on the dynamic course of immune changes in various phases of depression are important to consider. These include the (1) immunophysiology of COX enzymes, (2) nuance of cytokine signaling and (3) reported phase-specific involvement of the immune system in depression. Firstly, COX enzyme immunophysiology is incompletely understood [78]. Evidence from rodent studies suggests that by-products of COX-2 metabolism (e.g. prostaglandin D2 and docohexanoic acid) exert anti-inflammatory effects, therefore it can be argued that selective COX-2 inhibitor NSAIDs may increase neuroinflammation, Th1 immune responses and glial cell activation [46]. COX-1 is predominantly pro-inflammatory [46]. Taken together, non-selective COX-inhibitor NSAIDs would theoretically be more effective than COX-2 inhibitors [78], however, there are no quality trial exploring non-selective COX inhibitors.

Secondly, blanket blockade of pro-inflammatory cytokine signaling may not be advisable [22]. For example, TNF-α receptors have mostly opposing functions, i.e. Receptor 1 primarily mediates pro-inflammatory/neurodegenerative activities, whereas Receptor 2 is primarily involved in neuroprotective processes [22]. The Janus-faced signaling pathways of TNF-α make an argument against anti-TNF-α therapies; and indeed TNF-α inhibitors have small to negligible antidepressant effects as per the Kohler et al. publication [79]. A deeper understanding of cytokine biology and pathway-specific therapeutic manipulation is needed.

Thirdly, the phase of depression during which anti-inflammatory drugs are applied prompts further thoughts. While the onset of an episode and certain symptoms of depression appear well explained by this inflammatory model, the

underpinnings of the episodic and progressive nature, as well as relapse and remission status in depression require attention [29]. There is an emerging clinical and basic science evidence to suggest a phase-specific profile of immune-mediated dysfunction [29]. Anti-inflammatory pharmacotherapy may need to be used according to the phase of illness, and may need to be tailored based on the immune profile of the patient [29]. There is evidence to suggest anti-inflammatory treatments used outside the acute clinical phase may be detrimental [29]. As part of such a phase-specific model of inflammatory changes in depression it is still to be established whether anti-inflammatory agents only work, if peripheral inflammation is established in patients. Most of the studies in the field have not measured the state of inflammation before commencement of the anti-inflammatory trial with the aim to stratify patients for presence and absence or degree of inflammation. Further research is required to address this important gap in clinical research in depression-associated inflammation.

4.2 Potential Side-Effects of Anti-Inflammatory Agents

In addition to the above reasons for caution of un-reflected and premature clinical use of anti-inflammatory drugs, possible side-effects of anti-inflammatory drugs need further consideration. The current state of the literature suggests that the occurrence of potential side-effects on the use of NSAIDs needs further consideration, especially when considered as preventive treatments on large population scales. NSAIDs are among the most commonly used agents in clinical practice. They are employed as anti-inflammatory, analgesic and antipyretic agents for a wide spectrum of clinical conditions. Their anti-inflammatory properties are primarily due to inhibition of prostaglandin synthesis. Acute CNS toxicity related to NSAID use is pervasive and varied. A prospective study looking at ibuprofen overdose noted that 30% of patients experience CNS effects ranging from drowsiness to coma. Case reports have identified numerous neurologic sequelae including ataxia, vertigo, dizziness, recurrent falls, nystagmus, headache, encephalopathy and disorientation. Seizures have also been reported, mostly after overdose ingestions, but even therapeutic doses have occasionally been associated with seizures. One of the important neurologic side-effect attributed to the use of NSAIDs is aseptic meningitis. The clinical signs of drug-induced meningitis are similar to those of infectious meningitis and include fever, headache, photophobia and stiff neck. The laboratory findings are also similar, including cerebrospinal fluid (CSF) pleocytosis of several hundred or thousand cells, mainly neutrophils, elevated levels of protein, normal or low glucose levels and negative cultures. Drug-induced meningitis is a transient disorder with an excellent prognosis.

Most or all drugs used for the treatment of headache, including NSAIDs, may cause a condition known as medication overuse headache – a refractory chronic daily headache that tends to resolve following discontinuation of the analgesics. Reye's syndrome is a rare severe illness strongly associated with aspirin use.

Aspirin, the classic and most commonly used NSAID, has a well-documented effect in inhibiting intravascular clotting, thus reducing the occurrence of ischemic strokes and other vascular events. NSAIDs, however, have a double impact on coagulation. On the one hand, most agents inhibit the synthesis of thromboxane in the platelets, thereby inhibiting coagulation. On the other hand, they also inhibit the production of prostacyclin by endothelial cells, resulting in a prothrombotic state. Selective inhibition of COX-2 by drugs such as rofecoxib (Vioxx) and valdecoxib (Bextra) results in specific inhibition of synthesis of prostaglandins participating in inflammation and can lead to vascular complications including an increased risk for stroke [80].

5 Conclusions and Future Directions

Although clinical depression has long been associated with inflammation, limited treatment options have resulted from this immune-associated pathophysiology of depression. The literature suggests that an increased inflammatory response may define a subgroup of individuals in ultra-high-risk states, in acute disease episodes and of those with severe mental illness. Among anti-inflammatory agents and NSAIDs specifically, the selective COX inhibitor celecoxib shows the most evidence for antidepressant effects; however, the body of accumulated evidence on the role of selective and non-selective COX inhibitors is small and not suited to give clinical guidance at this stage. One could even argue that clinical proof-of-concept studies are still required before entering a phase of larger clinical trials. An improvement of future study methodologies would include the measure of the inflammation status of patients entering the trial. Future study results may point then to an indicated anti-inflammatory treatment based on the inflammation status of patients with clinical depression at commencement of the intervention. This might particularly apply to depressed patients with – but not limited to – concomitant inflammatory conditions such as rheumatoid arthritis, psoriasis and other chronic inflammatory conditions. Moreover, if anti-inflammatory treatment proves to be useful in acute depression, it is clinically worthwhile exploring whether this treatment has potential for treating also more severe forms of depression in the acute stage, and whether it is useful for relapse prevention. While the consideration of the phase of the illness is important for developing a potential phase-specific treatment approach using NSAIDs, the literature clearly points to the dynamic nature of the immune response in depression [29] and shows an involvement of both the innate and adaptive immune system in depression [81]. A focus on inflammation only might be shortsighted. While it has been shown that inflammation can be detrimental to the neurobiology of the brain, it has also been suggested that inflammation may lead to an increase in impaired neuroprotective mechanisms; hence a concept of enhancing neuroprotection plus a reduction in inflammation may be an extended avenue for future interventions. While progress has been made in the understanding of the role of inflammation in depression and first anti-

inflammatory treatments show some potential clinical value, the field is far away from an indicated and evidence-based approach for the use of anti-inflammatory agents in depression-associated inflammation. Clearly, an in-depth clinical study program including proof-of-concept studies is required to enhance the knowledge before NSAIDs can clinically be recommended as suitable agents for the treatment of symptoms in depression-associated inflammation.

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Does Diet Matter? The Use of Polyunsaturated Fatty Acids (PUFAs) and Other Dietary Supplements in Inflammation-Associated Depression

Caitlín N.M. Hastings, Hannah Sheridan, Carmine M. Pariante, and Valeria Mondelli

Abstract An increasingly pertinent issue in psychiatry in recent years is that of the limitations of conventional antidepressants, which are not effective in a large number of patients with major depressive disorder (MDD). Coupled with emerging hypotheses about the role of inflammation in depression, it would appear that it is time to look for alternative treatments for these symptoms.

This review will examine an emerging area in psychiatry, that of dietary supplements and the diet in general to treat depressive symptoms, and inflammation in depression. In particular, polyunsaturated fatty acids (PUFAs), probiotics and folic acid are three supplements that demonstrate the ability to target inflammation and other underlying systems in depression. While there is a definite need for more research in all these supplements to determine true efficacy, dosage and target populations, they can be used as mono- or adjunctive therapies to good effect, and show superior safety profiles when compared with more traditional alternatives.

Keywords Depression • Diet • Folic acid • Inflammation • Probiotics • PUFAs

Contents

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1 Introduction

While conventional antidepressants have been one of the most notable psychiatric developments since the mid twentieth century, the increase in limitations reported among depressed populations cannot be ignored. Worryingly, figures suggest that only a modest proportion (almost one-third) of patients ever achieve remission with monoamine antidepressant therapy alone [1]. As a consequence, a number of novel therapeutic approaches have been considered in order to ameliorate depressive symptoms, including changes in lifestyle and diet. Developing treatments targeted towards the heterogeneity of depression is not only required for improved quality of life among individuals with major depressive disorder (MDD) but remains an important aspect of translating more recent findings, produced from the *brain-mind-body trichotomy*, into clinical practice [2]. Diet, polyunsaturated fatty acids (PUFAs) and other dietary supplements in particular have been shown to address this multifaceted origin of depression by targeting multiple biological systems, including the inflammatory system, which has recently been recognized as crucially involved in this disorder.

Since MDD is a multifactorial disorder, several mechanisms are likely to underlie its aetiology. Thus, a number of hypotheses have been put forward in an attempt to elucidate its origin. The inflammatory hypothesis initially titled the macrophage theory of depression [3], or more recently, the malaise or cytokine theory of depression, has increasing relevance for MDD [4–8]. In short, the bidirectional language between the immune system and the central nervous system (CNS) is thought to be responsible for depressogenic changes caused by an upsurge of pro-inflammatory signalling molecules in certain areas of the brain [8, 9].

According to a widely accepted model, pro-inflammatory cytokines released as a result of stress, or as a direct consequence of immune activation, can cause disruption to monoamine metabolism, neuroendocrine function, synaptic plasticity, glutamate signalling and neurogenesis [4, 10, 11]. Moreover, direct evidence in favour of a causal relationship has been demonstrated by the administration of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, tumour necrosis factor alpha (TNF-α) and interferon alpha (IFN-α) [12]. In particular, these findings have resulted in an increase in depressive-like symptoms, such as low mood, anxiety, fatigue, anhedonia, cognitive dysfunction and disturbed sleep [4, 13].

Given findings in support of the *inflammatory hypothesis*, associations between inflammatory status and treatment response have opened up the possibility of producing reliable inflammatory biomarkers to improve upon treatments for this heterogeneous disorder [14, 15]. Emerging is the notion that attenuating inflammatory-mediated processes provides relief from depressive symptomology [7, 16–19]. Similarly, work conducted in our laboratory has found that higher

baseline gene expression of IL-1β and TNF-α predicts antidepressant resistance, thus these findings could have translational value for those who do not respond to traditional antidepressants [20]. While the use of anti-inflammatory strategies in the context of depression has obtained most attention, a putative anti-inflammatory and antidepressant effect could be delivered also by dietary supplements that regulate the immune system. Is there any evidence that dietary supplements with anti-inflammatory action can be beneficial for MDD, through inflammatory modulation?

Upon answering this question we will review the findings for different classes of dietary supplements throughout this chapter, with a view to obtain a greater understanding of the pharmacological concepts underlying the mechanisms of these agents, their efficacy and, finally, their relevance to MDD.

2 Dietary Supplements in Depression

Epidemiological studies have consistently demonstrated that diet plays a huge part in overall well-being, both mental and physical. It has also been shown in recent years that the gastrointestinal tract (GI) and the brain are closely related systems, with the GI system able to improve memory and learning, reduce anxiety and regulate stress levels [21]. This bidirectional relationship between the GI tract and CNS has been referred to as the 'brain-gut-axis' [22]. The high co-morbidity between stress-related psychiatric disorders, such as anxiety and depression, and gastric disorders, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), further strengthens this association [21]. Patients with MDD can also display altered GI function, increased oxidative stress and abnormal nutritional status with deficits in multiple micronutrients, which demonstrates that nutrition must be considered in treatment strategies by clinicians managing these patients [23].

Dietary supplements in general demonstrate a good safety profile, with minor adverse effects, especially when compared to other antidepressants and anti-inflammatory pharmacological alternatives. Hence they could be recommended as a safer treatment strategy, when indicated. Supplements can work in tandem with conventional antidepressants to help lessen their more unwanted side effects. For example, some antidepressants may increase blood pressure, and PUFAs may help to counteract these symptoms as they have been suggested to have anti-hypertensive effects [24]. Moreover, SSRIs have been associated with changes in bone metabolism, with an increased risk of hip fracture in females and loss of bone mineral density [25, 26], whereas folic acid has been proposed as a supplement to help to decrease fractures and so could be considered in tandem with a more traditional approach [27]. However, the most important use of dietary supplements is by a synergistic action in improving the antidepressant effects of other compounds, mainly through an anti-inflammatory action.

At present, the majority of clinical research related to inflammation in depression has been conducted on PUFAs, and so, this topic will encompass the largest

portion of this review. However, consideration will also be given to probiotics and folic acid, which may prove, in coming years, to be as valuable.

2.1 Polyunsaturated Fatty Acids

PUFAs are omega-3 (n-3) fatty acids derived entirely from dietary sources [28], and include eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) [29]. Their main function is to act as an essential component of membranes, maintaining their integrity and fluidity, and also to give rise to eicosanoids, which affect inflammation [30]. Eicosanoids from n-3 fatty acids reduce the synthesis of arachidonic acid (AA)-derived eicosanoids that are pro-inflammatory, and it is the balance between these fatty acids which maintains homeostasis in the immune system (as discussed in [16]). Indeed, consumption of n-6 PUFAs increases the production of pro-inflammatory cytokines, such as IL-1, TNF-α and IL-6, and consumption of n-3 reduces the activity of these n-6 acids, therefore reducing the production of these cytokines [31, 32]. Moreover, inflammatory processes in the central immune system are partly mediated by microglial activation, and n-3 acids inhibit constitutive and lipopolysaccharide (LPS)-induced microglial activation, pro-inflammatory cytokine production and cyclooxygenase (COX) expression [29]. The therapeutic use of n-3 acids for inflammatory conditions has long been known, and EPA can be used therapeutically in hypertension, diabetes, psoriasis, eczema, coronary heart disease, atherosclerosis and cancer [30].

Epidemiological Studies

In the last century, the Western diet has shifted away from n-3 rich foods, such as fish and flax seed, and is now high in n-6 foods, such as soy and corn oils, with ratios as high as 17:1 reported in favour of n-6 foods, instead of 1:1 in the case of animals and prehistoric humans [33]. This large dietary change may partly contribute to the concomitant rise of inflammatory diseases in modern society, such as cardiovascular disease (CVD), and depression [34].

Epidemiological evidence demonstrates that dietary fish consumption (a major source of n-3) reduces the risk of MDD, seasonal affective disorder (SAD), bipolar disorder and post-partum depression [29]. In an analysis by Hibbeln [35], a strong negative correlation between worldwide fish consumption and rates of MDD was reported from a cross-national depression database. Specifically, this has been demonstrated in the Mediterranean diet, where there is a decreased prevalence and incidence of depression, and the consumption of oily fish is higher [36]. This finding has also been reproduced in parts of Asia, like Japan and Korea, where the diet is higher in n-3 rich seafood. For example, a study by Yoshikawa et al. [37] found that, in a relatively small sample of 500 Japanese participants, increased fish consumption was associated with resilience to depression. Another small study by

Park et al. [38], with Korean participants and controls, demonstrated that the increased consumption of seafood and elevated erythrocyte levels of n-3 were associated with a decreased risk of depression.

Contradictorily, some studies have shown more mixed results, with no significant evidence that n-3 consumption reduces risk of depression/depressive symptoms. For example, a longitudinal study with more than 50,000 female participants between the ages of 50–77, and free from depressive symptoms at baseline, did not show a protective effect of n-3, obtained from a fish diet, on depression risk [39]. The same was also found in a study with almost 30,000 men in Finland, between the ages of 50–69 years, where no association was found between dietary intake of n-3 from fish and depressed mood, major depressive episodes or suicide [40]. These epidemiological studies are limited however, as they do not allow for causality or for certainty on n-3 intake from diet, as this is mainly taken from self-reported measures.

A role of endogenous PUFAs levels in the pathogenesis of depression has also been supported by a few studies that have specifically measured them in the blood of depressed patients. For example, a recent study by Chang et al. [41] has looked at the levels of n-3 in patients with CVD, and discovered that in sufferers with moderate depression (Hamilton Rating Scale for Depression (HAM-D) score more than 19) there were lower levels of DHA, n-3 and n-6:n-3 ratio than the non-depression group. This finding is mirrored in the general MDD population where lower concentrations of EPA and DHA are shown, with higher ratios of n-6 to n-3 acids when compared with healthy controls [42, 43]. A cross-sectional study correlating depressive symptoms with peripheral serum fatty acids and oxidative stress markers in females found that the depression scores were negatively correlated with concentration of n-3 acids, and positively correlated with IL-6 [44]. Two within-subjects studies found that lower baseline erythrocytes DHA levels and lower DHA plasma levels were correlated with an increased risk of developing depression during IFN-α treatment (a pro-inflammatory cytokine) [45]. A study looking at enzymes which metabolize PUFAs (Phospholipase A2 (PLA2) and cyclooxygenase 2 (COX2)) also found interesting results [46]. In a sample of patients with hepatitis C, the effects of seven single nucleotide polymorphisms in COX2 and PLA2 genes on the development of depression during IFN-α treatment were examined. A subsample of the patients was assessed for the erythrocyte levels of DHA, EPA and AA. It was found that genetic variations in COX2 were associated with lower DHA levels, and PLA2 variations were associated with lower EPA levels. Both increased the risk of IFN-α-induced depression, with the PLA2 Banl GG polymorphism associated with more somatic depressive symptoms. Considering this evidence, it is not surprising that many studies have looked at the potential antidepressant effects of PUFAs, either in the natural diet or as experimental supplementation.

Clinical Trials of PUFAs

PUFAs have been used successfully in clinical trials looking at improving mood in depression and other psychiatric conditions, and it may be wise to use this more robust evidence when considering their efficacy.

N-3 fatty acids have been shown to be successful when used as an adjunctive therapy [47]. This double-blind placebo-controlled trial compared 9.6 g of PUFAs or placebo in addition to usual treatment for 4 months. The sample receiving PUFAs had a longer remission rate and performed better on the majority of outcome measures when compared with the placebo group. In a study by Nemets et al. [48] 2 g of EPA were added to antidepressant medication for patients with MDD, all of whom demonstrated baseline scores of 18 or greater on the HAM-D. After 4 weeks, the mean reduction in the HAM-D scores was 12.4 points in the EPA group, compared with 1.6 in the placebo group. These results imply that n-3 may boost the antidepressant effects of traditional medication.

Moreover, meta-analyses which looked at n-3 fatty acid monotherapy trials in patients with MDD have noted a beneficial effect over placebo for the treatment of depressive symptoms [49–51]. Of note is the first double-blind placebo-controlled trial using PUFAs as a monotherapy in antenatal depression, which found that significant differences between placebo and n-3 were only seen after 6 weeks, implying that we may need longer trials to note efficacy [52]. Monotherapy has also been used to good effect in a paediatric population. A small pilot study examined 20 children between the ages of 6–12, who had suffered from depression for an average of 3 months. The placebo-controlled trial lasted just over 4 months. The treatment cohort received 400 mg of EPA and 200 mg of DHA daily, and 7 out of 10 children had a greater than 50% reduction in the Children's Depression Rating Scale (CDRS), when compared to 0 out of 10 children achieving this in the placebo group [53].

However, the success of these supplements does seem to be associated with the level of EPA, rather than DHA [16, 49, 54]. The addition of solely EPA to a conventional antidepressant was first examined by Puri et al. [55] in a therapeutic case study where it was shown that the dietary supplement had a positive effect on depressive symptoms. This may also explain the heterogeneity in the results of some clinical trials, which can sometimes be contradictory [56]. It has also been noted that the more severe the symptoms and the level of n-3 deficiency at the outset of treatment, the more dramatic the reduction of symptoms over time [49]. There is also evidence indicating that females may be more sensitive to n-3, with higher rates of intake predicting lower depressive symptoms [57, 58]. The dosage of EPA would also appear to be important, as there has been variance amongst trials when this has been examined [42]. However, most studies seem to indicate that the minimum effective dose is approximately 200–2,200 mg of EPA/day [49, 51]. A final point is the one raised by Lesperance et al. [59] that small significant effects may be overestimated. In their trial they found a marginal statistically significant difference between using 1,050 mg/d of EPA and 150 mg/d of DHA versus placebo,

but the clinical benefit was minimal. However, there was a benefit for patients with depression without co-morbid anxiety. The reporting of clinical impact would need to be considered when other studies are reporting their findings. Overall, the data suggests that PUFAs do indeed have an impact on mood, but more research is needed to determine the level of EPA vs. DHA, dosage, treatment duration and what patient population it is most effective in.

Potential Effects on Stress- and Inflammation-Related Mechanisms in Depression

Some experimental studies have examined the potential mechanisms by which PUFAs exert an antidepressant effect. The ratio of n-6 to n-3 acids has been demonstrated to influence physiological stress responses in both depressed patients and healthy populations. For example, a study looking at the effects of supplementation with n-3 PUFAs on adrenal activation after an induced stressor (mental arithmetic and Stroop's test) in healthy men found that 3 weeks of fish oil intake resulted in elimination of stress-induced cortisol release and dampened increases in epinephrine [60]. This finding suggests that supplementation for individuals with MDD could have great impact in reducing their stress responses.

Most studies mentioned support the idea that n-3 acids hold anti-inflammatory properties and help to relieve depressive symptoms: but is this reduction in depressive symptoms due to a reduction in inflammation? A preclinical study using the olfactory bulbectomised rat model of depression demonstrated that rats which were fed with EPA, rather than the control sham diet, showed significantly decreased behavioural changes in the open field test and improved spatial memory compared with controls, indicating a normalization of behavioural changes induced by stress (increased locomotor and rearing activity, and impaired memory in the Morris water maze). They also showed reduced corticotrophin-releasing factor (CRF) expression and corticosterone and IL-1β secretion [61].

Clinical trials have also been conducted, which appear to give good evidence to the theory that PUFAs could be a recommended therapy specifically for treatment of inflammation in depression. In a 2-week, double-blind, placebo-controlled trial comparing EPA, DHA and placebo in 162 patients receiving IFN-α, it was found that the incident rates of IFN-α induced depression were significantly lower in those treated with EPA, but not DHA, which echoes the findings mentioned earlier in this chapter, although they both delayed the onset of depression versus placebo [62]. In another double-blind trial, 155 participants with depression were randomized to receive EPA, DHA or placebo, and their baseline biomarker data was also captured. It was found that subjects with high levels of IL-1ra, IL-6, high-sensitivity C-reactive protein (hs-CRP) or leptin were more likely to respond to EPA and also reported the greatest decrease in HAM-D scores [63]. Other populations with increased baseline inflammation may benefit from PUFAs supplements. This could be true, for example, for old-age subjects, since pro-inflammatory cytokine production is increased after menopause or andropause even without the presence

of infection, stress or trauma. In a study examining the ratio of n-6:n-3 and depressive symptoms and pro-inflammatory cytokine synthesis in adults aged 40–86 (mean age of 66.67) it was found that higher levels of pro-inflammatory cytokines were associated with an increase in depressive symptom severity and also higher n-6:n-3 ratios [64]. Other conditions associated with chronic sub-threshold inflammation such as obesity, which is also strongly associated with increased depression risk [65], would need to be considered by future research with respect to PUFAs supplementation. This is in the context of the strong evidence showing that supplementary treatment with n-3 can help to balance inflammation and reduce the incidence of mood dysregulation.

In conclusion, the main benefit of PUFAs, and the reason to continue pursuing larger scale randomized controlled trials (RCTs), is their favourable safety profile and lack of adverse reactions, including reactions with concomitant medications. For instance, the most common adverse effect reported for PUFAs is gastrointestinal disturbance [66]. Although, the possibility of some supplements containing toxic levels of mercury or vitamin A must be taken into consideration, this risk is negligible in reality. As a result, these supplements would be highly appropriate as a treatment of depression for paediatric populations and also during pregnancy [52]. Moreover, it must be noted that throughout this section the issue of heterogeneity between trials has been mentioned. This issue was discussed further in a meta-analysis conducted by Bloch and Hannestad [67], where they found significant heterogeneity and publication bias in the 13 studies they examined. Worryingly, evidence for the benefits of n-3 was removed when publication bias was adjusted for using the trim-and-fill method. This supports the need for greater attention to the design of RCTs to get a clearer picture of the efficacy of n-3. In this context, the observation that greater efficacy was present when participants showed more severe baseline depression symptoms should be further examined and explored in future research.

2.2 Probiotics

Probiotics, which have been coined ‘psychobiotics’, are defined as a live organism which, when consumed, can have beneficial effects for those suffering from psychiatric disorders [68]. The use of probiotics in psychiatric research is still in its infancy, its use as an adjunct therapy in depression being first proposed by Logan and Katzman [23]. Moreover, it has been shown that certain bacteria produce neurochemicals relevant to depressive symptoms; for example, strains of *Lactobacillus* and *Bifidobacterium* secrete gamma-aminobutryic acid (GABA), an inhibitory neurotransmitter, which is implicated in anxiety and depression, and this has been suggested to have an effect on the *brain-gut axis* [69]. Other bacteria have also been shown in mice to affect serotonin plasma levels and to produce dopamine and norepinephrine [70, 71]. The *brain-gut axis* is connected predominantly via the

vagus nerves which perform a direct connection between the brain and gut and transmits all hormonal, neuronal and bacterial changes from the intestines [72].

Clinical Studies

The disorder that has received the most treatment with probiotics to date has been IBS, which is well documented to be associated with depression and anxiety. This has been studied with successful results in both the disease-specific and mood symptoms, and also with a parallel reduction in pro-inflammatory cytokines in the blood [73, 74]. In a study with sufferers of chronic fatigue syndrome (CFS) consuming a probiotic, it was found that there was a significant improvement in anxiety [75]. Two-month supplementation with probiotics also demonstrated improvements in inflammatory markers and oxidative stress in pregnant women and patients with type 2 diabetes mellitus [76, 77]. This improvement in well-being was also echoed in a study with healthy subjects consuming a probiotic yogurt for a 3-week period [78]: the third of the sample with the lowest baseline mood reported themselves happy rather than depressed after the study was completed. Moreover, in a study by Mohammadi et al. [79] consuming a probiotic yogurt or multispecies probiotic supplement for 6 weeks showed improvements in mental health.

To date, there has been one RCT conducted which examines the effects of probiotics on depression and inflammation. This study by Akkasheh et al. [80] examined 40 patients with MDD receiving a probiotic supplement (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidum*) or placebo for 8 weeks. The authors found significantly decreased Beck Depression Inventory (BDI) scores, oxidative stress and serum hs-CRP concentrations for the participants receiving the probiotic when compared to the placebo group. In addition, while there is a need for more large-scale placebo-controlled trials with probiotics in depression, there have been other studies that have produced some interesting results. For example, a double-blind, placebo-controlled randomized parallel group study using healthy human volunteers for 30 days showed that probiotic consumption reduced psychological distress on several scales (Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale (PSS) and the Coping Checklist (CCL), and reduced urinary free cortisol (UFC) levels [81].

Potential Mechanisms and Preclinical Studies

As previously mentioned, depression is associated with the presence of biomarkers of inflammation, and in rodent studies it has been shown that stress alters gut-barrier function, with leaked products stimulating Toll-like receptors (TLRs) and resulting in the production of inflammatory cytokines [82]. This has also been demonstrated in rhesus monkeys. Postnatal stress, induced by the separation of the infants from their mothers, altered the microbiota and reduced *Bifidobacterium* and

Lactobacillus levels [83]. Dinan et al. [68] suggested that this might partly explain the pro-inflammatory phenotype, which has been discussed above. The intestinal microbiota also performs an integral role in the immune system; the interaction between the gut mucosa and microbiota balances the production of pro-inflammatory cytokines such as IL-8, IL-1, IL-10 and TGF-β [84]. A preclinical study with rats treated with *Bifodobacteria Infantis* for 14 days, and submitted to the forced swim test, found that there were no behavioural effects [85]. However, there was a significant attenuation of IFN-γ, TNF-α and IL-6, and an increase in plasma concentrations of tryptophan (a serotonin precursor) and kynurenic acid when compared with controls. Moreover, in another study, rats separated from their mother were placed separately in two groups, and treated with *Bifodobacteria Infantis* or citalopram [85, 86]. Plasma cytokine levels, brain monoamine levels and central and peripheral HPA hormone levels were measured, and depressive behaviours were examined using the forced swimming test. Treatment with the probiotic was found to reverse depression-like behaviour and to decrease peripheral IL-6.

Interestingly, the immunoregulatory action of probiotics is proposed to work in the same manner as conventional antidepressants [87]. For example, traditional antidepressants have been suggested to, in addition to their actions on the serotonergic system, suppress inflammation by increasing production of the cytokine IL-10 [88]. Probiotics also increase levels of IL-10, but it is unknown if this increase in IL-10 has any effect on mood [89, 90]. Likewise, successful antidepressant treatment affects the HPA-axis in depressed patients, and hyper-response of the HPA-axis found in germ-free mice can be reversed with the use of a probiotic, and in these animals it is also found that levels of norepinephrine (NE) and 5-HT are also significantly reduced [91].

In conclusion, probiotics may prove to be a viable treatment for depression due to their psychoactive and anti-inflammatory properties, changing the gut-barrier function and ultimately reducing depressive symptoms. Indeed, Dinan et al. [68] suggests antibiotics such as minocycline have been shown to have an effect on depressive symptoms and also on inflammation by altering the microbiota. This further strengthens the probability that changes in the gut and inflammation may influence the mood. Other research has also shown that probiotic fortified laboratory chow increases the tissue levels of n-3, which is important given the effects of n-3 demonstrated above [90]. Probiotics may also favour the formation of folate, the effects of which will be discussed in further detail below [92]. This points to the possibility that dietary supplements could be used in tandem to boost each other's effects. The promising research presented in this section merits further investigation with more full-scale randomized placebo-controlled trials examining the effects of probiotics on inflammation in depression. Various strains of probiotics have been used to date, and it is still unknown which the most effective are. Probiotics also have a good safety profile with few side effects and may work well with other treatments for depression. This would be especially relevant if antibiotics such as minocycline are used more regularly in future for the treatment of inflammation in depression. They may help to balance any effects which

minocycline would have on intestinal flora while helping to boost the antidepressant effects.

2.3 *Folic Acid*

Folic acid, or folate, is a B vitamin and must be supplied through dietary consumption in order for humans to meet their daily requirements. Foods such as beans and legumes, leafy green vegetables and oranges are good sources. It is necessary for the synthesis and repair of DNA and as a co-factor in enzymatic reactions [93]. It has also been shown that folic acid deficiencies can be associated with psychiatric conditions such as depression, irritability, altered sleep and altered cognitive functioning, with these conditions responding to folate therapy [94–97]. Lower folate levels in patients with depression have also been linked to lower rates of treatment response to pharmacological interventions [98]. This was shown in a study with depressed geriatric patients with low folate levels taking either sertraline or nortriptyline. They found that the higher baseline folate levels predicted greater treatment response on the Profile of Mood States (POMS); a self-report scale [99].

It has been proposed that folic acid supplementation may result in an antidepressant-like effect, with a reduction in norepinephrine secretion and increased serotonin activity [100, 101]. This would tie in with folate's importance in the methylation of homocysteine, for its conversion to *S*-adenosyl-methionine (SAMe), which has been shown to influence serotonin metabolism and have some antidepressant effects [102]. However, a meta-analysis looking at RCTs using folic acid as an augmentation to antidepressants found that its use may be effective, but would not suffice as a replacement for conventional antidepressants. They were also unable to assess whether this effectiveness was particularly evident in those with low folate, as the studies had not taken a baseline reading from participants [103]. Therefore, endogenous levels of folate would need to be considered in future research.

Folate has also been found to have an impact on the immune system, or rather, a deficiency in folate has been found to be associated with higher levels of pro-inflammatory cytokines. Folate levels are a determinant for plasma homocysteine, and increased levels of homocysteine are considered a marker for folate deficiency [104]. High levels of homocysteine make endothelial cells more prone to injury, which in turn leads to inflammation in the blood vessels and this may act as a risk factor for atherogenesis, coronary artery disease and stroke [105]. It has also been shown that folic acid treatment for hyperhomocysteinemia reduced the levels of both homocysteine and pro-inflammatory cytokines [106]. In a study looking at B-vitamin deficiency as a risk factor for vascular disease, it was found that in vitro folate deficiency in mouse cells (achieved by growing the mouse monocyte cell line RAW264.7 under folate restriction) increased the release of pro-inflammatory cytokines IL1-β, IL-6, TNF-α and MCP-1 two to threefold [107]. This study also showed that nitric oxide (NO) production was attenuated by folate deficiency and,

in contrast with other findings, these changes were independent of the concentration of homocysteine. This last study suggests that folate levels rather than homocysteine are responsible for attenuation of inflammation.

To date, there have been few studies linking the anti-inflammatory effect of folic acid with mood changes in depression. One study by Resler et al. [108] compared adjunctive therapy of folic acid or placebo with fluoxetine. Patients receiving folate had a significantly lower depression score on the HAM-D after 6 weeks than the placebo group, and also plasma homocysteine was significantly decreased, with a significant negative correlation between homocysteine and folate. Although this only provides indirect evidence, a reduction in homocysteine levels is likely to influence immune function, as discussed above. Finally, it has been shown that folic acid can enhance concentrations of n-3 acids, which again can have an anti-inflammatory action, as discussed earlier in this section [109].

In conclusion, while folate has been found to have some effects on inflammation and on depressive symptoms, further research is needed to determine whether these effects on depression could be associated with a reduction in inflammation. It would also be important to measure baseline levels of folate as it has been shown that this may have an effect on the improvement in depressive symptoms.

3 Final Remarks and Future Research

For future consideration, there is great potential to design and implement novel treatments for depressive subtypes. The dietary supplements discussed in this chapter have been shown to work well in conjunction with traditional approaches, and also on their own, but they should also be considered in tandem with one another, as all of these compounds seem to directly or indirectly have an anti-inflammatory action: this symbiosis ought to be considered in future trials. Inflammatory biomarkers offer a gateway towards personalized medicine, and as a result not only will we be better equipped to understand the mechanisms underpinning MDD, but we may also be able to predict response rates for the novel agents described.

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Role of Neuro-Immunological Factors in the Pathophysiology of Mood Disorders: Implications for Novel Therapeutics for Treatment Resistant Depression

Anindya Bhattacharya and Wayne C. Drevets

Abstract Mood disorders are associated with persistently high rates of morbidity and mortality, despite the widespread availability of antidepressant treatments. One limitation to extant therapeutic options has been that nearly all approved antidepressant pharmacotherapies exert a similar primary action of blocking monoamine transporters, and few options exist for transitioning treatment resistant patients to alternatives with distinct mechanisms. An emerging area of science that promises novel pathways to antidepressant and mood-stabilizing therapies has followed from evidence that immunological factors play major roles in the pathophysiology of at least some mood disorder subtypes. Here we review evidence that the compounds that reduce the release or signaling of neuroactive cytokines, particularly IL-1β, IL-6, and TNF-α, can exert antidepressant effects in subgroups of depressed patients who are identified by blood-based biomarkers associated with inflammation. Within this context we discuss the role of microglia in central neuroinflammation, and the interaction between the peripheral immune system and the central synaptic microenvironment during and after neuroinflammation. Finally we review data using preclinical neuroinflammation models that produce depression-like behaviors in experimental animals to guide the discovery of novel neuro-immune drug targets.

Keywords Depression • IL-1β • IL-6 • Microglia • Mood disorders • Neuroimmunology • Neuroinflammation • P2X7 • TNF-α

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Contents

Mood disorders constitute clinically pleomorphic syndromes consisting of behavioral and experiential changes in the emotional, cognitive, visceral, and appetitive domains that show moderate to high heritability, but remain idiopathic with respect to etiology. The main mood disorders, major depressive disorder (MDD) and bipolar disorder (BD) show relatively high lifetime prevalence rates [1] and despite the availability of many antidepressant drugs, MDD is ranked by the World Health Organization (WHO) as the highest global cause of "years of life lived with disability" for all age groups. The persistence of this global public health problem partly reflects the limited efficacy of extant therapies, as about one third of MDD patients do not achieve remission despite multiple trials using different treatments, while another third experience illness relapse and recurrence despite continued adherence to initially effective treatments [2–4]. One limitation of extant antidepressant pharmacotherapies is that they essentially all target biogenic amine based mechanisms, so that for patients who do not respond to such mechanisms, therapeutic options with distinct mechanisms have been largely unavailable.

Notably, the results of studies that compared depressed patients who respond to monoamine reuptake inhibiting agents versus those who do not consistently have shown that the non-responders manifest abnormal elevations in a variety of pro-inflammatory immunological markers [5–9]. These data converge with evidence that factors within the innate and the adaptive immune system play roles in the pathophysiology of MDD and BD, potentially thereby illuminating new targets for novel therapeutics in mood disorders [10, 11]. As reviewed below the findings that administration of pro-inflammatory cytokines such as interferon-alpha or low dose endotoxin can induce depressive symptoms in a subset of humans who have not previously been depressed [12], along with the implication of immune pathway dysregulation by genome-wide association studies (GWAS) of primary mood disorders, suggest that some individuals have a biological diathesis to manifest depressive symptoms under immune challenge [13, 14]. Such conclusions have been corroborated pre-clinically by similar phenomena, specifically by showing that immune activation produces depression-like behaviors in repeatedly stressed animals and that these behaviors can be prevented or reversed by anti-inflammatory treatments [15]. Similarly, an emerging clinical literature provides evidence that

some types of anti-inflammatory treatments can produce antidepressant effects in depressed patients with peripheral blood evidence of inflammation [16].

1 Interplay of the Immune System and the Central Nervous System (CNS)

The emerging neuro-immunological literature suggests that immune cells in the periphery and/or the brain interact with neurons in the CNS to play roles in the pathophysiology of mood disorders [11, 17]. These data point to the existence of a bi-directional immune-connectivity between the peripheral and central compartments [18–20]. The interplay of the immune system and the CNS involving pro-inflammatory cytokines, chemokines, and related molecular processes that lead to microglial activation and astrogliosis is referred to as *neuroinflammation*. However, in the CNS the biological concomitants of an inflammatory state differ in many respects from conventional inflammation involving peripheral immune cells [21]. Thus the neuroimmunology field has broadened in perspective to also encompass the mechanisms by which the peripheral immune system modulates central neurophysiology. In contrast, the neuroinflammatory changes in microglia, astrocytes, and oligodendrocytes that putatively contribute to the causal mechanisms underlying multiple sclerosis, Parkinson's disease, and epilepsy are generally absent in mood disorders. For example, post mortem studies of glial cell function, structure and density do not show the astrocytosis and amoeboid microglial morphology that is manifest in multiple sclerosis, trauma, or neurodegeneration. Instead such studies have demonstrated reductions in oligodendroglia, impaired astroglial function, and intermediate morphologies of activated microglia [22]. One exception to this general set of findings in post mortem studies of mood disorders involves elderly patients characterized by a *late age of depression onset*; such patients show clinical and neuropathological evidence for a pathophysiological process mediated via cerebrovascular disease, including astrogliosis, inflammation, and other histopathological correlates of ischemic disease [23]. Nevertheless, debate remains whether neuroinflammatory processes play pathological or adaptive/compensatory roles in the pathophysiology underlying early onset mood disorders, which instead have been associated with a combination of genetic and environmental (e.g., early life trauma) risk factors [24–26].

In the CNS, bone marrow derived immune cells have a restricted access due to an intact blood–brain barrier (BBB) and blood–CSF barrier. During an injury or infection when this barrier is compromised, peripheral immune cells can penetrate the CNS causing neuroinflammation. Nevertheless, other conditions exist in which macrophages and monocytes from the periphery can migrate into the CNS [27, 28] and the CNS lymphatics may serve as conduits of peripheral to central cellular migration [29, 30]. In addition, and probably more pertinent to the topic of neuroinflammation, microglia constitute the critical cell types that change from a

"surveillance" mode to a "response" mode during injury and disease pathology. Resting microglia manifest a distinct "ramified" morphology whose function is to sense the local environment and maintain homeostasis among the neurons, astrocytes, and oligodendrocytes that participate in synaptic function [31] and transmission. During pathology associated with neuroinflammation, microglia respond by adopting an amoeboid morphology and release gliotransmitters such as pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IFN-γ), chemokines, glutamate, ATP, nitric oxide, and reactive oxygen and nitrogen species that alter the neuro-glia functional interactions [32]. Microglial activation also involves up-regulation of cellular markers, increased microglial proliferation and migration, and a shift in structure and function towards an "M1" pro-inflammatory phenotype. The M1 phenotype occurs in response to tissue injury, stress, and infection as part of the adaptive immune response which ultimately leads to reparative processes. The repair is mediated by microglia, predominantly of the anti-inflammatory (M2) phenotype, which are more phagocytic in nature. Nevertheless, microglia more commonly exist in a range of phenotypes that are intermediate in morphology between the M1 and M2 [33–35]; the differential roles of M1/M2 microglia and their role in CNS (patho)physiology are reviewed elsewhere [36, 37]. Notably, post mortem assessment of brain tissue from patients with MDD and BD revealed that the microglia manifest such an intermediate "activated" morphology associated with greater quinolinic acid expression (implying pro-inflammatory activation of the kynurenine pathway) in the subgenual anterior cingulate cortex area consistently implicated in the pathophysiology of mood disorders [38].

One type of phagocytic activity performed by the microglia involves synaptic pruning that regulates interneuronal connectivity and restores the optimal multipartite synaptic function from the altered states that arise during neuroinflammatory states [39–41]. Thus in mood disorders it has remained unclear whether microglial activation manifests as a reparative response or instead comprises a pathological mechanism that initiates disruption of normative neurophysiology [42]. In other neuropathological conditions, the extant data suggest that neuroinflammation can play pathological roles under some conditions and adaptive/restorative roles in others, with both roles potentially co-existing within the context of a particular CNS disease states. Nevertheless, chronic and/or dysregulated neuroinflammation eventually contributes to a pathological phenotype within the CNS. For example, the extant preclinical and emerging clinical data suggest that glial factors released from microglia and astrocytes during neuroinflammation modulate synaptic plasticity and neurogenesis and impact the neurocircuitry in a manner that can manifest behaviorally in much of the symptomology that defines mood disorders.

Peripheral immune cells also play roles in CNS function that include supporting learning and memory [43–48], protecting against pathogens (e.g., as evidenced by IFN-γ-mediated control of *Toxoplasma gondii* [49], and inducing neuropathology (e.g., in multiple sclerosis). The peripheral immune system also can play a beneficial or healing role in CNS pathology. For example, a controlled amplification of the autoimmune response was associated with improved neuronal survival in rodent models of acute CNS injury [50] and chronic neurodegeneration [51, 52]. The

complex interplay between the peripheral immune system and the CNS in mediating beneficial components of the immune response to CNS pathology is only beginning to be elucidated.

Conversely, changes in peripheral immune cell populations have also been associated with CNS pathologies that do not feature clear penetration of the blood–brain or blood–CSF barrier by circulating cells. Pro-inflammatory cytokines released during peripheral infection are associated with behavioral correlates of depressive mood—termed sickness behavior [53]. Links between sickness behavior and a tryptophan metabolizing enzyme, namely indoleamine 2,3 dioxygenase (IDO), have been demonstrated [54], establishing a potential association between cytokines and monoamine deficiency.

Moreover, post-traumatic stress disorder (PTSD), a syndrome characterized by chronic anxiety, depression, and hyperarousal arising in the aftermath of traumatic stress constitutes a condition that links CNS pathology, stress, and immune system dysregulation at the level of inflammatory cascades and gene networks. For example, in soldiers studied before and after deployment in areas of active conflict, CD14+ monocyte-associated factors were differentially regulated in PTSD sufferers [55]. Notably monocytes are primary producers of the neuroactive cytokines IL-6, IL-1β, and TNF-α, which have been linked to mood disorders in preclinical studies and clinical populations (reviewed elsewhere in this volume). CD14+ monocytes are mobilized into circulation primarily by CCL2, a chemokine produced by glial [56] and blood–brain barrier cells [57] during neuroinflammation. These findings implicate a link between glial activation and loss of peripheral immune homeostasis leading to chronic feedback between the CNS and periphery in PTSD.

2 Abnormalities in Immunological Factors in a Subset of Patients with Mood Disorders Suggest Novel Antidepressant Targets for Such Subgroups

A rapidly expanding scientific literature suggests that alterations in immune system function and neuroinflammation play major roles in the pathophysiology of at least some subtypes of mood disorders [17, 58–61]. This evidence has encouraged targeted and rational drug discovery efforts with a view to intervene using immune modulating treatments for mood disorders [16]. Immune mediators for which the mean concentrations are increased in the blood and cerebrospinal fluid (CSF) of patients with mood disorders versus healthy controls, both when assessed at baseline and after exposure to stressors, include IL-6, IL-1β, IFN-α, TNF-α, prostaglandin E2, and the chemokine CCL2 [62–66]. The mRNA transcripts for these cytokines and for other related innate immune system genes have also been elevated in peripheral blood cells in patients with mood disorders relative to healthy controls matched for age, BMI, smoking, and comorbid medical conditions [6, 22,

67–70]. The clinical significance of these findings is supported by evidence that the elevations of these cytokines in the plasma or CSF of patients with MDD or BD relative to controls are correlated with illness severity and/or suicidality ratings (reviewed elsewhere in this volume). Moreover, *successful* response to conventional antidepressant drugs is associated with reductions in these cytokine levels in depressed patients, although *non-response* to conventional antidepressants is predicted by higher IL1-β, IL-6, and CRP levels in the pre-treatment baseline [6, 9, 71]. The preliminary evidence reviewed below suggests that in depressed patients who manifest both resistance to monoamine reuptake inhibitor antidepressant agents and elevation in pro-inflammatory cytokines/acute phase proteins, certain classes of anti-inflammatory agents can produce antidepressant effects.

The relationship between immune challenge and the development of "sickness behavior" as well as other more clearly pathological depressive symptomatology is instructive in considering the etiology of mood disorders. In one of the clearest examples indicating that elevated cytokine signaling can cause depressive symptoms, immune challenge with interferon-α (IFN-α) during the treatment of hepatitis C or other medical conditions reliably has induced the major depressive syndrome (and less commonly manic symptoms) in 30–40% of previously non-depressed humans [64]. This neuropsychiatric sequelae of IFN-α converges with other types of evidence to suggest that elevated signaling of some neuroactive cytokines can play a causal role in inducing depressive symptoms [72]. Within the days following IFN-α administration, previously non-depressed patients show a behavioral complex that includes anorexia, fatigue, lower mood, reduced social interaction, and reduced engagement in pleasurable activities, a symptom complex referred to in the research literature as "sickness behavior" [73]. Notably, in the subset of patients who receive IFN-α who go on to develop major depressive episodes, more specific depressive symptoms such as pessimism, anxiety, and suicide ideation arise later than the initial appearance of sickness behavior, and the likelihood of developing an MDE continues to rise with longer time spent receiving IFN-α [74]. Thus the symptoms of the major depressive episode differ from those of sickness behavior by magnitude in some cases (e.g., more severely depressed mood and pervasive anhedonia in MDE) and by quality in others (e.g., pathological anxiety and suicide ideation). In addition there is also evidence that IFN-α induced depression differs from depression arising in medically healthy MDD subjects by the presence of pathological guilt in the latter, but not in the former condition [75, 76]. These data are notable within the context of evidence that primary MDD is heterogeneous, with subgroups that are distinguishable on the basis of clinical symptomatology as well as immunological markers [58], as reviewed below.

The prevailing hypothesis holds that IFN-α induced major depressive episodes constitute an example of a "pro-inflammatory state induced mood disorder" that manifests in some individuals exposed to IFN-α on the basis of a biological predisposition. A corollary to this hypothesis posits that a subset of the "primary" MDD population also manifests depressive symptoms due to the influence of elevated neuroactive cytokine signaling caused via other etiologies. An example of the existence of a biological predisposition toward IFN-α induced depression

was provided by the report of a single nucleotide polymorphism (SNP) in the IL-6 receptor gene that resulted in lower IL-6 expression, and also was associated with decreased susceptibility to the development of depressive symptoms during IFN-α treatment [77].

Notably, some patients who manifest IFN-α induced major depressive episodes improve during SSRI treatment, leading physicians to prophylactically initiate SSRIs in the weeks prior to initiating IFN-α for some patients [78]. Of the symptom domains affected by IFN-α, however, the depressed mood symptom dimension is most responsive, whereas the anxiety, cognitive and neurovegetative symptoms appear less responsive (or unresponsive) to prophylactic SSRI treatment [79]. These observations hold intriguing therapeutic implications, because in patients with primary mood disorders, higher blood levels of proinflammatory cytokines or their mRNA transcripts predict non-response to treatment with SSRIs or other conventional antidepressants [6–9]. It is conceivable that depressed patients who both manifest chronic inflammation and prove nonresponsive to conventional antidepressant drug treatment may benefit from immune modulating treatments.

The hypothesis that anti-inflammatory agents may exert antidepressant effects in depressed patients has been tested both in patients who have primary MDD and in *patients with autoimmune disorders who manifest clinically significant depressive symptoms* [16]. For example, patients with psoriasis who received the anti-TNF-α agent etanercept showed significant improvement in depressive symptoms in response to drug versus placebo as assessed using conventional depression rating scales, and this difference was evident earlier than the associated changes in pain or skin lesions [80], implying the antidepressant response occurred independently of psychological benefits related to the improvement in the skin lesions per se. In this study patients treated with etanercept also had significant improvements in fatigue. Notably, while the improvements in fatigue correlated with decreasing joint pain, the improvements in depression were less correlated with objective measures of skin clearance or joint pain.

3 Immunological Biomarker Data from Mood Disordered Samples Shows Heterogeneity That May Hold Therapeutic Implications

In studies of *primary MDD patients* treated using anti-inflammatory agents, the extant data suggest that subgroups characterized by high levels of pro-inflammatory biomarkers are most likely to benefit (see below). This observation appears intuitive when the findings that mean concentrations of cytokine levels are elevated between depressed and control samples are considered in further detail. The distribution of the immunological data from these studies suggests that the differences reported in *mean values* are attributable to a subset(s) of the depressed patients. This observation appears consistent with accumulating evidence of biological and genetic

differences between subtypes of depressed subjects with MDD, who otherwise appear phenotypically homogeneous in many aspects of symptom presentation. For example, from the Netherlands Study of Depression and Anxiety database, Lamers et al. [58] used subgroups defined initially using cluster analysis of depressive signs and symptoms, and then further differentiated these subtypes based on serum protein profiles. The identified analytes consisted largely of inflammatory (e.g., CRP) and metabolic markers (e.g., insulin), supporting the conceptualization of a subtype(s) characterized by metabolic disturbances and inflammation. These researchers [81] also showed that these subgroups appeared stable across time, with patients moving between different levels of severity, but not between subtypes, during longitudinal follow-up. In another example, data from the Mood Inflame Consortium identified three MDD subtypes: one manifest in MDD patients aged $\geq$28 years that was characterized by *increased* expression of monocyte genes and decreased expression of glucocorticoid receptor (GR) α versus β subunit ratio, a second in MDD patients <28 years of age who showed a severe course of depression (characterized by recurrent type, illness onset <15 years of age, history of childhood trauma, and prominent panic/arousal symptoms) but monocyte gene expression similar to healthy controls, and a third also manifest in MDD patients <28 years of age characterized by a milder illness course (most with first episode of depression, age at onset $\geq$15 years, and absent panic symptoms) that exhibited a strongly *reduced* inflammatory monocyte activation compared to controls [82].

Within the bipolar spectrum of mood disorders, another study from the Mood Inflame Consortium identified a biomarker signature composed of multiple immunological factors that discriminated the majority of BD patients from healthy controls. Using whole-genome expression profiling of RNA obtained from purified CD14+ monocytes, Padmos and colleagues reported elevated mRNAs of inflammatory (e.g., TNF, PDE4B, IL-1β, IL6, TNFAIP3), trafficking, survival (e.g., BCL2A), and mitogen-activated protein kinase pathway (e.g., MAPK6, ATF3) genes in BD subjects in various illness phases, as well as in affected offspring of BD parents [69]. Notably, in peripheral blood mononuclear cells (PBMC) from the same subjects assessed via fluorescence-activated cell sorting (FACS) analysis, the percentages of anti-inflammatory CD4+CD25highFoxP3+ regulatory T cells were higher in BD patients <40 years of age, while percentages of Th1, Th2, and Th17 cells were normal. Together these results thus showed enhancement of both pro-inflammatory monocyte and anti-inflammatory T cell mediators in BD [83].

4 Novel Drug Targets at the Crossroads of Neuroimmunology and Mood Disorders

With continued and refined understanding of the role of immune cells and their mediators in the periphery and the CNS, it is anticipated that new mechanisms will be discovered that can exert antidepressant and mood-stabilizing effects in primary

mood disorders. Several comprehensive reviews have highlighted potential drug targets in neuroimmunology for mood disorders [11, 21, 84]. In this chapter, we summarize evidence that highlights TNF-α, IL-6, and IL-1β signaling in the pathophysiology of mood disorders.

TNF-α: TNF-α signaling appears to play a major role in mood disorders [85]. In meta-analyses of clinical studies, plasma TNF-α correlated with depression severity and level of resistance to conventional antidepressants [62]. A causal relationship between TNF-α elevation and depressive symptoms was suggested by observations that in patients with immunological diseases such as rheumatoid arthritis and psoriasis, anti-TNF-α treatment alleviates depressed mood; as reviewed above, these antidepressant effects do not appear attributable simply to improvement in sickness symptoms, such as fatigue, or in the underlying autoimmune disorder [86]. Consistent with these observations, the TNF-α receptor 1, TNF-α receptor 2, and TNF-α knockout mouse models all show antidepressant-like phenotypes [87, 88]. Likewise, systemic administration of antibodies targeting TNF-α in chronic models of stress reversed the anhedonic behaviors, suggesting that TNF-α signaling contributes to depressogenic behaviors in rodents [89, 90].

Nevertheless, a clinical study of the efficacy of infliximab (a monoclonal antibody against TNF-α) in depressed patients generated negative results on depressive symptoms rating using a conventional depression rating scale [91]. A *post hoc* investigation of data from this study, however, revealed a significant positive correlation between clinical improvement and pre-treatment levels of the nonspecific inflammation marker, CRP, raising the possibility that antidepressant effects may be limited to individuals who manifest a pro-inflammatory diathesis. Nevertheless, because the test of the *a priori* hypothesis in this study was negative, the question has remained whether targeting TNF-α via large molecules introduced in the periphery alone can produce an antidepressant effect (since very low proportions of peripherally administered monoclonal antibodies enter the brain following acute treatment), or whether therapies that reduce TNF-α signaling must instead directly engage targets in the CNS.

IL-6: In studies of MDD or BD one of the more highly replicated biomarker abnormalities has been an elevation in peripheral blood IL-6 concentrations [92]. Notably during IFN-α treatment the magnitude of the increase in plasma and CSF IL-6 levels correlates positively with depressive symptom severity. Conversely, the above-mentioned functional polymorphism in the promoter region of the IL-6 gene (rs1800795) that results in decreased IL-6 expression is associated with a significantly lower risk for developing major depressive episodes during IFN-α treatment [77]. The relationship to IL-6 function is compatible with findings that, in patients with primary mood disorders higher IL-6 levels in the CSF correlated with suicidality, and elevated IL-6 levels in the plasma correlated with non-responsiveness to conventional antidepressant drugs [93]. In contrast, during the euthymic (i.e., asymptomatic) phase of BD, the CSF concentration of IL-6 was decreased with respect to healthy controls, despite the same BD subjects showing an abnormal elevation in the CSF levels of IL-1β [65].

Although IL-6 can be released by immune cells in the CNS as well as in the periphery, preclinical evidence suggests that elevated IL-6 release from peripheral immune cells is sufficient to induce depressive behaviors, irrespective of central immune system activation. In studies conducted by Hodes and colleagues [15] to elucidate the biological basis of susceptibility to depression-like behaviors under stress, mice that developed a persistent depression-like phenotype in response to social defeat stress (SDS) were compared to genetically identical mice that did not develop depression-like behaviors under SDS. The susceptible animals differed from the resilient animals by showing elevated basal IL-6 levels in the pre-SDS condition and higher IL-6 release in response to the stressed condition. In addition, white blood cells sampled pre-SDS from susceptible mice showed higher LPS-induced IL-6 release *ex vivo* compared to cells from resilient mice. Crucially, the susceptibility to the depression-like phenotype could be altered toward either susceptibility or resilience by generating bone marrow chimeras that had hemopoetic stem cells transplanted from high IL-6 expressing mice or IL-6 knock-out mice, respectively. The bone marrow recipients in these studies had received radiation to their bodies while the head was shielded, so the hemopoetic stem cells in periphery conferred the susceptibility to the depression-like phenotype under stress.

IL-1β: In contrast to the therapeutic potential offered by neutralizing IL-6 predominantly in the periphery, the extant data suggest that for the pro-inflammatory cytokine IL-1β, reducing signaling in the brain may prove critical to achieving antidepressant effects. IL-1β is probably the most potent pro-inflammatory cytokine released from microglia in the brain. Clinical studies found that IL-1β is present at abnormally higher levels in plasma, CSF, and postmortem brain tissue of individuals with mood disorders, and that IL-1β levels correlated positively with depression severity [63, 65]. Anisman and colleagues reported increased IL-1β production from lymphocytes in patients with dysthymic disorder and a modest correlation existed between the cytokine and depressive symptoms [94]. In studies of primary mood disordered subgroups, IL-1β has been linked with both geriatric depression and postpartum depression [95, 96].

In animal models of stress-induced depression-like behaviors, several groups showed that IL-1β signaling is critical to the acquisition of the depression-like phenotype [97, 98]. The development of the depressive behavioral phenotype during chronic stress can be blocked by IL-1 receptor antagonists, and is absent in IL-1R receptor knockout mice [99]. In addition, manipulation of central IL-1β, either by exogenous administration of IL-1β directly into the brain, or by selective ablation of signaling via pharmacology or genetics, produced behavioral analogues of depression when IL-1β was increased, or antidepressant-like effects when IL-1β was decreased [100]. The IL-1β driven changes in the brain resulted in decreased neurogenesis in the hippocampus [99] and increased corticosterone response to stress in the periphery [101], suggesting an interplay between stress-induced-IL-1β release and HPA axis function.

Recently, it was shown that both acute and chronic stress increase brain IL-1β release [102] [100]. Stress-induced IL-1β release appears to be driven by

ATP-induced activation of the P2X purinoceptor 7 ion channel (P2X7), and genetic deletion of P2X7 receptors results in antidepressant-like reversal of stress-induced depressogenic behaviors in rodents [102, 103]. Other experimental evidence has similarly demonstrated that P2X7 activation causes release of IL-1β ([104]). The initiation of transcription and translation of the pro-form of IL-1β is induced by activation of Toll-like receptor (TLR), but it is the second signal from P2X7 (due to ATP activating the ion channel) that results in maturation and release of the pro-inflammatory IL-1β cytokine; this process has been referred as a "two-hit" model of IL-1β release. Priming of the TLRs is achieved by factors such as cellular debris, by endotoxins, by damage- and pathogen-associated molecular pattern molecules (DAMPs and PAMPs, respectively). Since P2X7 is abundantly expressed in blood cells, IL-1β release in the blood has been used as a biomarker of P2X7 activity in both preclinical and clinical assessment of target engagement.

Based on robust microglial expression of P2X7, and IL-1β signaling leading to neuroinflammation, CNS penetrable P2X7 antagonists would be potentially beneficial for treating mood disorders, and there is growing evidence that strengthens the role of P2X7 in MDD and BD. Several human genetic studies have associated the highly polymorphic *P2RX7* gene with the risk for developing both BD and MDD, and some of these mutations have been linked to a modulation of P2X7 channel function *in vitro* [105, 106]. The rs2230912-G allele exhibits a gain-of-function and human monocytes expressing this variant secreted more IL-1β in response to activation of P2X7 than monocytes expressing a wild-type variant [107]. It is conceivable that such a variant in P2X7 receptors based in human microglia would lead to enhanced IL-1β release (or production), leading to neuroinflammation over time. Nevertheless, several other GWAS studies have not confirmed the association between *P2RX7* variants and the risk for mood disorders [108], so the relationship between the variation in P2RX7 and depression is not yet established. The lack of clarity for a genetic association of P2RX7 variation in the risk for mood disorders (or any disease phenotype) is perhaps not surprising as the underlying factors of such pathologies are often a result of interplay between genetic (often many genes), environmental, and developmental factors.

In addition to the human genetic literature, several laboratories have demonstrated that P2X7 knockout mice manifest a protective phenotype in models of depression and mania, strengthening the hypothesis that P2X7 antagonism may be therapeutically beneficial in mood disorders. Consistent with the antidepressant phenotype observed in P2X7 knockout mice, emerging data suggest that P2X7 antagonists can reverse depressogenic behaviors in animal models. For example, pharmacological antagonism of P2X7 (by AZ-10606120 and A-804598) restored the deficit observed in the preference for a sucrose solution (a putative behavioral analogue of anhedonia) induced by either chronic stress or systemic administration of lipopolysaccharide (LPS) administration [103]. Recently, it was shown that a P2X7 selective, brain-penetrant antagonist was efficacious in chronic stress models in rats [102]. In addition, a large corpus of evidence suggests that manipulation of central IL-1β (by either exogenous administration or selective ablation of signaling by pharmacological or genetic manipulation) results in depression-like behaviors

when IL-1β is increased, or in resilience against the development of depression-like behaviors when IL-1β is decreased [97, 99, 109]. These observations appear consistent with the above-mentioned findings that IL-1β levels are abnormally elevated in the plasma, cerebrospinal fluid (CSF), and postmortem brain tissue obtained from MDD and BD patients [63, 65, 110].

Preclinical data also suggest that P2X7 antagonism may produce anti-manic or mood-stabilizing effects in BD [111]. For example, P2X7 antagonism produced attenuation of amphetamine-induced sensitization of hyperactivity [112, 113], a putative rodent model of mania-like behavior, and similar phenotypes were observed in P2X7 knockout mice [111] suggesting a potential therapeutic role of P2X7 antagonism in the manic phase of BD. Taken together, it remains plausible that a selective and brain-penetrant P2X7 antagonist may be therapeutically beneficial in mood disorders, especially targeting treatment resistant patient populations.

5 Conclusion

Neuroimmunology stands at the interface of emerging biology and breakthrough therapeutics for mood disorders. Taken together, the extant data support the hypothesis that elevated cytokine levels contribute to the pathophysiology of depression and the neurobiological mechanisms underlying resistance to conventional antidepressant drugs, in at least a subpopulation of depressed patients. They also suggest that specific cytokines (such as TNF-α, IL-6, and IL-1β) and their effectors and regulators (such as P2X7) may constitute novel therapeutic targets for depression. However, the extant postmortem data also indicate that mood disorders are not associated with classical neuroinflammation, and in vivo blood-based biomarker studies suggest that not all patients suffering from mood disorders manifest an inflammatory component. Consequently, for clinical proof-of-concept studies with compounds that target signaling of microglia, astrocytes, or cytokines/chemokines, it may prove necessary to discriminate the patient population suffering from concomitant depression and neuroinflammation through the aid of immunological biomarkers.

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