

Bipolar Disorder: Role of Inflammation and the Development of Disease Biomarkers

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Bipolar disorder is a severe and enduring psychiatric condition which in many cases starts during early adulthood and follows a relapsing and remitting course throughout life. In many patients the disease follows a progressive path with brief periods of inter-episode recovery, sub-threshold symptoms, treatment resistance and increasing functional impairment in the biopsychosocial domains. Knowledge about the neurobiology of bipolar disorder is increasing steadily and evidence from several lines of research implicates immuno-inflammatory mechanisms in the brain and periphery in the etiopathogenesis of this illness and its comorbidities. The main findings are an increase in the levels of proinflammatory cytokines during acute episodes with a decrease in neurotrophic support. Related to these factors are glial cell dysfunction, neuro-endocrine abnormalities and neurotransmitter aberrations which together cause plastic changes in the mood regulating areas of the brain and neuroprogression of the bipolar diathesis. Research in the above mentioned areas is providing an opportunity to discover novel biomarkers for the disease and the field is reaching a point where major breakthroughs can be expected in the not too distant future. It is hoped that with new discoveries fresh avenues will be found to better treat an otherwise recalcitrant disease.

Psychiatry Investig 2016;13(1):18-33

Key Words Bipolar disorder, Inflammation, Biomarkers, Cytokines, Neurotrophins.

INTRODUCTION

Bipolar disorder (BD) is a chronic medical condition with the usual onset in adolescence and early adult life.¹ It is marked by recurrent mood exacerbations which can be of opposite polarity, ranging from major depressive episodes to manic episodes. In many patients the disease is characterized by a progressively severe course with frequent episodes, incomplete inter-episode recovery, sub-threshold affective symptoms and refractoriness to treatment measures.² This is accompanied with impairment in biological, psychological and social functioning of the patients. The bipolar diathesis exerts a pervasive effect and causes adverse consequences for all aspect of life. BD has a very high heritability of approximately 80% and the disease represents a complex interplay of genes and environment.³ Life expectancy of bipolar patients is short-

ened by 10 to 15 years not just because of an increased suicide rate but by the presence of such medical comorbidities as cardiovascular disease, diabetes and other metabolic conditions.⁴

Knowledge about the neurobiology of bipolar disorder is expanding rapidly and an increasing body of evidence points to the fact that inflammatory processes play a major role in the pathophysiology of this condition.⁵ The later include aspects of innate and adaptive immunological responses which cause damage to the central nervous system and the peripheral organs. The inflammatory immune responses result in such manifestations as neuroprogression of the disease as well as accelerated atherosclerosis, dyslipidemia, insulin resistance and premature mortality.⁶

In the past few years several studies have documented increased levels of circulating proinflammatory cytokines in different phases of bipolar disorder.⁷ These are polypeptide molecules secreted by macrophages, T lymphocytes and endothelial cells and sub serve such diverse functions as activation of neutrophils, proliferation of B cells, synthesis of acute-phase proteins, and increased vascular permeability. Examples of cytokines include interleukins (ILs), tumor necrosis factors (TNFs), interferons (INFs), transforming growth factors (TGFs) and chemokines. Cytokines can gain access to the CNS where they activate microglia which are one of the three

Received: January 6, 2015 **Revised:** May 21, 2015

Accepted: June 18, 2015 **Available online:** November 20, 2015

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types of glial cells, the others being astroglia and oligodendrocytes.⁸ The microglia act as resident macrophages in the brain and participate in inflammation in response to injury, infection or insult to the CNS. In the acute phase these cells act to contain neuronal damage but if activated chronically as happens in repeated mood episodes of bipolar disorder the homeostatic balance is overwhelmed and there exists a constant inflammatory environment which causes damage to the neurons.⁹ As a result, there is failure of neuronal function because of decreased synptogenesis, dendritic loss, oxidative stress, mitochondrial dysfunction and ultimate activation of the apoptotic cascade leading to death of the neurons.¹⁰

CYTOKINE MARKERS AND PERIPHERAL INFLAMMATION IN BIPOLAR DISORDER

Recent studies have fully demonstrated the association between manic and depressive episodes and a pro-inflammatory state involving both the innate and adaptive immune system. See the table for a detailed overview of immunological abnormalities in bipolar disorder (Table 1). In particular, T-helper cells 1 (Th1) are known to mediate cellular immune reactions and include production of cytokines IL-1, IL-2, IL-6, interferon-gamma, and TNF-alpha. Th2 cells enhance antibody-mediated immune reactions and the production of cytokines IL-4, IL-5, and IL-10 while Th3 cells heighten production of TGF-beta. Elevated levels of TNF-alpha have been reported in manic and depressive episodes in bipolar disorder patients.²⁴ High levels of IL-6 and TNF-alpha have also been reported during mania with IL-6 levels returning to baseline after treatment with mood stabilizers while TNF-alpha continuing to remain high.²⁵ During mania IL-2, IL-6, IL-8 and INF-gamma were the increased pro-inflammatory cytokines, while only IL-6 was found to be increased during depression.²³ Moreover bipolar depression was characterized by an altered balance between IL-6 and the anti-inflammatory IL-10.²³ Conversely, elevated levels of the anti-inflammatory IL-4 were noticed in bipolar subjects in studies that did not utilize mitogen stimulation.³⁰ IL-4 induces transformation of naïve T-helper cells into Th2 cells and reduces production of Th1 cells and macrophages. As such IL-4 is a key switch regulating the balance between cellular and antibody-based immunity. It might be speculated that IL-4 elevation in bipolar disorder may be of a compensatory nature, to buffer against the increase of proinflammatory cytokines seen in the condition. The findings imply that mania and to a lesser extent bipolar depression, are associated with a pro-inflammatory state.²¹ Overall, the data suggest that successful treatment with mood stabilizers leading to a euthymic state may reverse

inflammation and normalize peripheral levels of inflammatory mediators.²⁰

Elevated IL-6 has been one of the most consistent findings in BD.¹⁶ IL-6 is a pleiotropic cytokine that is produced at sites of inflammation, secreted by T-cells and macrophages. The role of IL-6 is to stimulate the B and T lymphocytes and hepatocytes to produce acute inflammatory proteins such as high-sensitivity C reactive protein (hs-CRP). Furthermore, it has been suggested that cytokines such as IL-1, IL-6 and TNF-alpha act in a concerted manner augmenting the immune response and help in the elimination of pathogens and the resolution of the inflammatory challenge.³¹ On the other hand, inflammatory cytokines are a known cause of diminished sensitivity to glucocorticoid and insulin receptors.³² Combined with autonomic disturbance seen in bipolar disorder, increased platelet/endothelial aggregation and unhealthy lifestyle, elevated inflammation may contribute to substantially increased risk of cardiovascular disease.³³ The cumulative effect of hypercortisolemia as a result of impaired hypothalamic-pituitary-adrenal (HPA) axis regulation combined with compromised glucocorticoid and insulin receptor activity, aggravated by inflammatory cytokines might explain the high rate of metabolic syndrome, dyslipidemia and diabetes in the bipolar population.³⁴ In addition, increased peripheral inflammation has been associated with numerous symptoms of mood disorders such as malaise, fatigue, anhedonia, impairment of concentration, anxiety, irritability, social disconnection, hopelessness, suicidal ideation, bodily aches, and disturbance in sleep and appetite.³⁵

INFLAMMATORY MEDIATORS AND THE CENTRAL NERVOUS SYSTEM

Peripheral inflammatory signals can gain access to the CNS through several pathways including: 1) areas of the brain not covered by the blood-brain barrier (BBB), such as the circumventricular organ, 2) afferent vagal fibers may convey the peripheral cytokines and other inflammatory mediators to their nuclei, including nucleus tractus solitarius, 3) BBB cells have the ability to import cytokines via active transport, 4) peripheral immune cells such as macrophages, T-lymphocytes and monocytes may gain access to the CNS and release the inflammatory mediators, and 5) BBB cells such as endothelial cells and pericytes can be induced to release inflammatory signals. Elevation of cerebrospinal fluid (CSF) inflammatory cytokines such as IL-1 beta has been substantiated in bipolar patients, especially if they have experienced recent manic episodes, compared with healthy volunteers.³⁶

Inflammatory cytokines activate microglia in the brain causing them to intensify the inflammatory response by re-

Table 1. Circulating cytokine abnormalities in bipolar disorder patients

Study (year)	Sample	Measurements	Main findings	Interpretation
Bai et al. (2014) ¹¹⁾	130 BD, 149 UD, 130 NC.	Soluble interleukin-6 receptor (sIL-6R), soluble interleukin-2 receptor (sIL-2R), C-reactive protein (CRP), soluble tumor necrosis factor type 1 receptor (sTNF-R1), soluble p-selectin receptor (sP-selectin), monocyte chemoattractant protein-1 (MCP-1).	Higher levels of sIL-6R, sIL-2R, CRP, sTNF-R1, MCP-1 in BD subjects as compared to UD subjects and NC.	More severe inflammatory dysregulation in BD as compared to UD.
Bai et al. (2014) ⁷⁾	130 BD, 130 NC. Among BD subjects 77 had BD type I, 53 had BD type II; 75 in euthymia, 14 manic/hypomanic, and 41 in depressive state.	sIL-6R, sIL-2R, CRP, sTNF-R1, sP-selectin, MCP-1.	BD subjects had significantly higher levels of all cytokine as compared to NC. BD type II patients had significantly lower levels of sTNF-R1 than BD type I patients. Patients in a depressive state had significantly lower levels of sTNF-R1 than patients in manic/hypomanic and euthymic states.	Immune dysregulation in BD. sTNF-R1 may be a potential biomarker for different phases and types of BD.
Brambilla et al. (2014) ¹²⁾	20 chronic SCZ, 20 chronic BD, 20 NC.	Chemokines, chemokine receptors, cytokines, regulatory T-cell markers.	Classical monocyte activation (M1) markers IL-6, ccl3 significantly increased in BD as compared to SCZ and NC. Markers of alternative (M2) monocyte activation ccl1, ccl22, IL-10 coherently decreased in BD. T-cell markers – ccr5 down regulated and IL-4 up regulated in BD compared to NC. Down regulated ccl2 and TGF-beta in BD compared to SCZ and NC. All explored immune markers preserved in SCZ.	Coherent increased M1/decreased M2 signature in peripheral blood of BD patients with potential Th1/Th2 shift. Proinflammatory response in chronic BD as compared to chronic SCZ.
Barbosa et al. (2014) ¹³⁾	46 BD patients (23 in mania and 23 in euthymia), 23 NC.	IL-33 and its soluble receptor sST2.	IL-33 higher in BD patients; no difference in sST2 between BD and NC.	IL-33 is a cytokine with multiple functions and may act as a nuclear factor regulating transcription. BD is a multisystem condition with a proinflammatory profile.
Doganavsargil-Baysal et al. (2013) ¹⁴⁾	54 BD type I patients in euthymia, 18 NC.	TNF-alpha, sTNF-R1, sTNF-R2.	sTNF-R1 and sTNF-R2 levels higher in euthymic BD subjects than NC. No difference TNF-alpha.	Proinflammatory process continues in euthymic period in BD subjects.

Table 1. Circulating cytokine abnormalities in bipolar disorder patients (continued)

Study (year)	Sample	Measurements	Main findings	Interpretation
Hope et al. (2013) ⁽¹⁵⁾	192 SCZ, 130 BD	sTNF-R1, IL-1Ra, IL-6, vonWillibrand factor (vWF), osteoprotegerin (OPG). GAF and PANSS used to assess current symptom level in both groups.	After controlling for confounders, IL-1Ra and sTNF-R1 significantly associated with GAF and PANSS scores.	IL-1Ra and sTNF-R1 associated with both general disease severity and psychotic features. There is role of immune activation in the core pathological mechanisms of severe mental disorders.
Remlinger-Molenda et al. (2012) ⁽¹⁶⁾	76 BD, 35 with mania, 41 with depression; 78 NC	IL-6, IL-10, IL-1beta, TNF-alpha, IFN-gamma.	IL-6 higher during mania as compared to NC. IL-6 higher in mania than in remission. IL-10 higher in manic patients during remission as compared to NC. In manic subjects INF-gamma correlated with symptom severity. IL-6 correlated to intensity of manic state during remission. IFN-gamma higher in acute depressive episode as compared to remission, acute mania and NC. IFN-gamma higher in depressed patients during remission than NC.	Immune disturbances in BD. Either decreased or pathologically increased immune responses in BD. Cytokine profiles different in mania and depression. Clinical improvement connected with immuno-modulation process with changes in cytokine levels during remission.
Remlinger-Molenda et al. (2012) ⁽¹⁷⁾	121 BD, 35 in remission after mania, 41 in remission after depression and 45 in sustained remission of >6 months with lithium; 78 NC	IL-1beta, IL-2, IL-6, IL-10, TNF-alpha, IFN-gamma.	IL-10 higher in manic patients in remission. IFN-gamma higher in depressed patients in remission. Cytokine concentrations in patients with sustained remission no different from NC.	Sustained remission achieved by lithium maintenance brings cytokine status to level similar to NC.
Kunz et al. (2011) ⁽¹⁸⁾	20 euthymic BD, 53 chronic, stabilized SCZ, 80 NC	TNF-alpha, IL-6, IL-10.	IL-6 increased in SCZ compared to NC and BD. IL-6 no different in euthymic BD and NC. IL-10 higher in BD and SCZ than NC. TNF-alpha, no difference among the 3 groups.	Chronic immune activation in SCZ. BD, an episode-related inflammatory syndrome. TNF-alpha a treatment response marker. Different balance of pro and anti-inflammatory response in SCZ and BD.

Table 1. Circulating cytokine abnormalities in bipolar disorder patients (continued)

Study (year)	Sample	Measurements	Main findings	Interpretation
Hope et al. (2011) ¹⁹⁾	112 BD, 153 SCZ, 239 NC. BD and SCZ patients were grouped into elevated, depressed and neutral mood.	sTNF-R1, IL-1Ra, IL-6, CRP, OPG, vWF.	In BD all inflammatory markers lowest in depressed state. sTNF-R1 positively correlated with elevated mood. Sad mood negatively correlated with OPG, IL-6, IL-1Ra. Neutral mood group had significantly higher levels of OPG and IL-6 compared to NC. SCZ patients had no significant associations between cytokine levels and affective states.	Significant associations between affective symptomatology and inflammatory markers in BD but not SCZ. Immune mechanisms are linked to core psychopathology of BD.
Guloksuz et al. (2010) ²⁰⁾	31 euthymic BD-16 medication-free, 15 on lithium; 16 NC.	INF-gamma, TNF-alpha, IL-2, IL-4, IL-5, IL-10.	No differences in cytokine levels between medication-free BD and NC. TNF-alpha and IL-4 levels higher in lithium group compared to other 2 groups.	Proinflammatory state resolves in euthymia. Lithium influences cytokine profile which could create a confounding factor.
Barbosa et al. (2011) ²¹⁾	53 BD-34 in mania, 19 in euthymia; 38 NC.	TNF-alpha, sTNF-R1, sTNF-R2.	No difference in TNF-alpha, sTNF-R2 between groups. sTNF-R1 higher in BD. Manic patients had higher sTNF-R1 than euthymic patients and NC.	Proinflammatory status in BD patients during mania. Inflammatory mechanisms involved with the pathophysiology of BD.
Hope et al. (2009) ²²⁾	125 BD, 186 SCZ, 244 NC.	sTNF-R1, IL-1Ra, IL-6, CRP, vWF.	sTNF-R1 and vWF increased in BD and SCZ compared to NC. No major differences between BD and SCZ. Controlling for confounders like age, gender, ethnicity, cardiovascular disorders, kidney and liver function did not affect the results.	Factors related to endothelial function increased. Specific alterations of endothelium-related inflammation process in both BD and SCZ.
Britzke et al. (2009) ²³⁾	61 BD-23 in mania, 24 in depression and 14 euthymic; 25 NC.	TNF-alpha, IL-2, IL-4, IL-6, IL-10, IFN-gamma.	Proinflammatory cytokines IL-2, IL-4, IL-6 increased in mania as compared to NC. Only IL-6 increased in depression. No significant differences in cytokine levels between BD patients in remission and NC. Mood symptoms showed a positive correlation with IL-2 and IL-6.	Mania, and to a less extent, depression associated with a proinflammatory state. Changes in cytokine profile more pronounced during acute episodes than euthymia. Immune system needs to be investigated as a target for future treatment development.
Ortiz-Dominguez et al. (2007) ²⁴⁾	20 BD type I in manic or depressive phases; 33 NC.	IL-1beta, IL-2, IL-4, IL-6, TNF-alpha.	In depressed BD patients compared to NC IL-6 and TNF-alpha increased, whereas IL-2 decreased. In manic BD patients compared to NC IL-4 and TNF-alpha increased, while IL-1beta and IL-6 decreased.	There are phasic differences in the serum levels of cytokines in BD.

Table 1. Circulating cytokine abnormalities in bipolar disorder patients (continued)

Study (year)	Sample	Measurements	Main findings	Interpretation
Kim et al. (2007) ²⁵⁾	37 BD patients in mania, 74 NC.	Mitogen-induced production of TNF- α , IL-2, IL-4, IL-6, IFN- γ .	IL-6 and TNF- α production significantly higher in manic BD subjects as compared to NC. IL-4 values significantly lower in patients compared to NC. After 6 weeks of treatment, the levels of IL-6 significantly decreased compared to baseline.	Increased activity of proinflammatory cytokines in BD patients. Imbalance between proinflammatory and anti-inflammatory cytokines play a role in the pathophysiology of BD.
O'Brien et al. (2006) ²⁶⁾	BD patients in mania or depression compared to NC. Sample size small with all medicated patients.	IL-6, IL-8, IL-10, TNF- α , sIL-6R.	Significantly higher production of proinflammatory IL-8 and TNF- α in bipolar depression compared to NC. Manic patients also had increased plasma levels of IL-8 and TNF- α compared to NC. Anti-inflammatory IL-10 levels did not differ across the 3 groups.	Increased proinflammatory cytokine production in BD subjects in mania as well as depression. Acute BD episodes associated with an inflammatory state.
Kim et al. (2004) ²⁷⁾	70 BD patients in mania, 96 NC.	Th1 cytokine INF- γ , Th2 cytokine IL-4, Th3 cytokine TGF- β 1. Cytokine levels assessed on admission and 8 weeks after mood stabilizer treatment.	Plasma concentrations of IFN- γ and IL-4 significantly higher and TGF- β 1 lower in patients compared to NC. Cytokine ratios returned to control values after treatment.	TGF- β 1 modulates Th1 and Th2 cytokine production. TGF- β 1 plays a role in the pathophysiology of BD via modulation of Th2 function. There is immune imbalance in BD.
Su et al. (2002) ²⁸⁾	20 BD inpatients with mania, 15 NC.	Phyto-hemagglutinin stimulated production of INF- γ and IL-10.	INF- γ production significantly lower in acute mania and during remission as compared to NC. IL-10 no different during mania, remission and in NC.	Other major psychiatric disorders like MDD had different cytokine profiles. Immune modulation varies in patients with different psychiatric disorders.
Tsai et al. (2001) ²⁹⁾	31 BD in acute mania, 31 NC.	Plasma sIL-2R, sIL-6R examined during mania and in remission.	Plasma sIL-2R but not sIL-6R was significantly higher in acute mania than in subsequent remission and controls. sIL-2R levels significantly correlated with severity of mania and YMRS scores. Other variables like age, gender, age of onset, number of prior episodes and psychosis had no effect on cytokine values.	sIL-2R a trait marker in MDD and SCZ. sIL-2R a biological indicator of manic severity. Immuno-modulatory mechanisms vary in different major psychiatric disorders.

BD: bipolar disorder, UD: unipolar depression, SCZ: schizophrenia, MDD: major depressive disorder, NC: normal controls, GAF: global assessment of functioning, PANSS: Positive and Negative Syndrome Scale, YMRS: Young Mania Rating Scale, TNF: tumor necrosis factors, TGF: transforming growth factors, IL: interleukins

leasing reactive oxygen species, reactive nitrogen species, cytokines and chemokines.³⁷ This chemical milieu of oxidative stress and inflammatory signals precipitates a change in astroglial function. Glial indoleamine 2, 3-dioxygenase, a tryptophan metabolizing enzyme is up-regulated, resulting in greater production of neurotoxic kynurenine metabolites and quinolinic acid (QA) as opposed to 5HT synthesis.³⁸ In addition, altered astroglia diminish their neurotrophic production including brain derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) and start extruding cytokines and glutamate. Glutamate released from the astroglia accesses extra-synaptic N-methyl D-aspartate (NMDA) receptors, causing suppression of BDNF synthesis and activation of the proapoptotic cascade. QA is a potent NMDA agonist that may further potentiate excitotoxicity.³⁹

At supraphysiological levels proinflammatory cytokines can directly injure neurons. TNF-alpha, for instance interacts with two neuronal receptors, p55 (TNF-RI) and p75 (TNF-RII) and ligand binding to either receptor can activate an apoptotic signaling cascade. The TNF-R then links with the TNF receptor-associated death domain resulting in recruitment and internalization of Fas, activation of caspase-8, and cell death.⁴⁰

Proinflammatory cytokines increase the expression of serotonin and dopamine transporters, disrupting monoamine signaling. Furthermore, increased oxidative stress may compromise monoamine synthesis by depleting tetrahydrobiopterin, a key co-enzyme in monoamine production.⁴¹ Less efficient monoamine signaling is correlated with higher levels of CSF IL-6 and greater severity of depressive symptoms. In a group of suicidal patients that included several bipolar subjects elevated CSF IL-6 was associated with higher 5HT and dopamine turnover, as evidenced by increased level of their metabolites.⁴² In summary, immune dysregulation in BD is associated with alterations in monoamine and glutamate signaling, impaired neuroplasticity and neurotrophic support, contributing to the symptomatic expression of the disease and its comorbidities.

INFLAMMATION AND CHANGES IN NEUROPLASTICITY

The role of BDNF has received more attention in mood disorder research than other members of the neurotrophin family. It is involved in neuronal maturation, differentiation and survival, synaptic plasticity, and long-term memory consolidation.⁴³ There is compelling preclinical evidence which suggests that BDNF plays an important role in regulating the release of serotonin, glutamate, and gamma-amino butyric acid (GABA).⁴⁴ Furthermore, BDNF even acts as an immunomod-

ulator in the periphery of the body. There seems to be a bidirectional communication between the immune system and neuroplasticity regulators.⁴⁵ Recent preclinical research has identified microglia originated BDNF as a key contributor to neuronal tropomyosin-receptor-kinase-B (TrkB) phosphorylation and ensuing changes in synaptic plasticity. Thus, microglia BDNF release appears to have a central role in learning and memory-related synaptic plasticity.⁴⁶ BDNF is released from the neurons in two forms: as pro-BDNF (pBDNF) and its chemically abbreviated version, mature BDNF (mBDNF). These two molecules participate in opposing functions: pBDNF binds to p75 receptor initiating apoptosis or shriveling of neurons, whereas mBDNF has primary affinity for the TrkB receptor which mediates neuroplasticity and resilience.⁴⁷

Several studies have demonstrated decreased levels of BDNF in bipolar depressed and manic subjects and low levels of this factor are correlated with clinical severity of the episodes. Although diminished levels of BDNF have been reported in both treated and untreated patients, one study found normal levels of BDNF in euthymic, treated patients suggesting a potential neurotrophic benefit of pharmacotherapy.⁴⁸ A reciprocal relationship between BDNF and inflammatory mediators is an important marker of the progression of bipolar diathesis. Chronicity of bipolar illness, repeated episodes, and decreased responsiveness to treatment all have a synergistic impact on a decline of neurotrophic signaling and increase in inflammation. In the latter stages of BD an imbalance between inflammatory cytokines, in particular TNF-alpha, mediators of oxidative stress and BDNF persists even between mood exacerbations and is clinically manifested as incomplete inter-episode recovery, sub-syndromal symptoms, and rapid cycling.⁴⁹ With progression of the disease there are accompanying structural brain changes with decreased neural regeneration of the hippocampus, neuronal loss in key mood regulating areas and abnormalities in circuits connecting limbic and para-limbic regions.⁵⁰

NEUROENDOCRINE AND AUTONOMIC DYSREGULATION

Alterations in HPA axis function in bipolar disorder have been well substantiated.⁵¹ Exaggerated release of corticotrophin-releasing factor contributes to increased adrenocorticotrophic hormone secretion and a subsequent elevation of circulating cortisol. These disturbances are most likely attributable to deficits in cortico-limbic regulation with consequent amygdala over activity and a compromised hippocampal regulatory role.⁵² Moreover, glucocorticoid receptors appear to have diminished sensitivity in mood disorders possibly due to elevation of inflammatory cytokines, thereby disrupt-

ing physiological feedback regulation on the HPA axis and immune system.⁵³ Indeed, even euthymic bipolar patients show dysregulation of the HPA axis with reduced diurnal variation of serum cortisol and flattening of the cortisol curve. These abnormalities are further exacerbated in subjects with repeated mood episodes resulting in higher overall cortisol level which is an ominous indicator of compromised overall health.⁵⁴ Highlighting the relevance of these neuroendocrine anomalies, a recent study has associated elevated evening cortisol levels in bipolar individuals with a history of suicidal behavior.⁵⁵

In addition to HPA axis irregularities, bipolar disorder may be associated with excessive sympathetic nervous system (SNS) activity. For example, extra-neuronal norepinephrine was reported to be elevated in a group of bipolar patients relative to healthy controls.⁵⁶ Autonomic dysregulation, more generally reflected by decreased parasympathetic activity and elevated sympathetic activity may be a trait marker for bipolar disorder as indicated by a report of markedly lower heart rate variability in euthymic bipolar patients than in healthy controls.⁵⁷ The constellation of SNS over activity, parasympathetic withdrawal, glucocorticoid receptor insufficiency, and elevated inflammatory signaling may help account, at least in part for the increased risk of metabolic syndrome, endocrine disorders, and vascular disease seen in bipolar patients.⁵⁸ Underscoring the relevance of this pattern of neuroendocrine, autonomic and immune changes is the fact that vascular disease has recently been identified as the leading cause of excess mortality in bipolar disorder.⁵⁹

NEUROGLIAL ABNORMALITIES

Convergent evidence from several areas of research including histopathologic, biochemical and neuroimaging have provided clues to the abnormalities of all three of the glial cell families in bipolar disorder linking the pathogenesis of the condition to structural and functional anomalies in astroglia, oligodendrocytes and microglia.⁶⁰ Postmortem studies of bipolar patients have noted a reduction in both glial cell numbers and density.⁶¹ Glial cell abnormalities have been reported in the subgenual anterior cingulate gyrus, dorsolateral prefrontal cortex, orbitofrontal cortex and the amygdala of unmedicated bipolar patients. Interestingly, one study found evidence that treatment with lithium or valproate may mitigate some of the glial loss.⁶² Furthermore, a significant 29% reduction in oligodendroglia numerical density in the dorsolateral prefrontal white matter was detected in bipolar patients compared with controls.⁶³ Evidence of diminished myelin staining in the dorsolateral prefrontal cortex and reductions of S100B immune-positive oligodendrocytes in the hippocam-

pus of bipolar subjects further extend these findings.⁶⁴ Indeed, research evidence indicates that oligodendroglial deficits may be the key CNS cellular abnormality in bipolar disorder.⁶⁵

A postmortem study of suicidal bipolar, major depressive disorder and schizophrenic patients provides intriguing insights into a possible role of microglia in the pathophysiology of these conditions. Unlike mood-disorder patients who committed suicide, subjects who had the same diagnosis but died of other causes showed no evidence of brain microgliosis. However, suicidal mood disorder patients, including the bipolar group had a substantial elevation of microglia density in the dorsolateral prefrontal cortex, anterior cingulate gyrus and mediodorsal thalamus when compared with controls and mood-disorder patients who did not die of suicide. Given the established role of microglia in CNS inflammation, these findings raise the intriguing possibility that suicidality might literally be the consequence of disease flare-up.⁶⁶ Supporting the role for these microglia changes in disease pathology is the fact that a remarkable overlap exists between the sites of cellular pathology and the brain regions with altered structure and functioning in neuroimaging studies of bipolar illness.⁶⁷

In contrast to the evidence for a glial role in bipolar pathogenesis, the data supporting a role for a primary neuronal pathology in the condition are less convincing. With a few notable exceptions, neuronal changes in bipolar disorder are mostly morphological in character, possibly attributable to apoptosis and thinning of interneuronal neuropil, and are much less extensive than glial pathology. In summary, the available evidence does not provide much support for viewing bipolar disorder as a typical neurodegenerative disease. Unlike conventional neurodegenerative disorders, which are associated with marked neuronal loss and prominent gliosis, glial loss seems to be the dominant cellular pathology in bipolar illness. According to the available evidence, bipolar disorder is much more a disease characterized by glial pathology rather than being a neurodegenerative condition.⁶⁸ Although the clinical correlates of the cellular pathology in BD await better description, there is little doubt that documented histopathological changes in key cortico-limbic areas and the white-matter tracts play an important role in the clinical manifestations of bipolar illness.

ASTROGLIA FUNCTION AND GLUTAMATE ABNORMALITIES

Astrocytes which are frequently found ensheathing synapses are an essential part of this structure along with the presynaptic and postsynaptic neurons. This close proximity has given rise to the term “tripartite synapse”. An ongoing molecular

dialog between glial cells and neurons has an important role in the regulation of glutamate signaling. Glutamate released from neurons is taken up by astroglia and converted to glutamine before being returned to neurons as the “raw material” for further neurotransmitter synthesis.⁶⁹ In addition glutamine is a precursor for glutathione which serves as the main antioxidant in the brain. A magnetic resonance spectroscopic (MRS) study has indicated an elevation in glutamine/glutamate ratio in the anterior cingulate gyrus and parieto-occipital cortex of manic subjects compared with matched controls, pointing to excessive glutamatergic activity and/or aberrant glial/neuronal interactions in the context of bipolar illness.⁶⁹ Several MRS studies and a postmortem study have reported an increase in glutamatergic transmission in the frontal cortex and hippocampus of bipolar subjects relative to control groups.⁷⁰

Glx is the term used to denote glutamate and related compounds like glutamine, GABA and glutathione in the brain. MRS provides an opportunity for *in vivo* evaluation of glutamate-related metabolites and depending on field strength and signal-to-noise ratio, glutamate and glutamine can be quantified either separately or as a composite of Glx. Recent comprehensive meta-analyses have identified relatively consistent elevation of Glx in anterior cingulate gyrus, medial prefrontal cortex, dorsolateral prefrontal cortex, parieto-occipital cortex, insula and hippocampus. These findings persisted across the bipolar mood states and even in euthymic bipolar patients, relative to control group.⁷¹ Effect sizes related to Glx signal were more robust in mania and depression than in euthymic patients. It can be assumed that at least some of the glutamatergic aberration in bipolar disorder reflects functional and numerical glial abnormalities given their cardinal role in the regulation of glutamate metabolism and signaling.⁷² Distribution of aberrant Glx signals in bipolar disorder also substantially overlaps with glial alterations reported in postmortem cytological studies. Anatomical structures characterized by anomalous MRS signals in bipolar disorder are some of the key components of the cortico-limbic regulatory pathways involved in regulation of mood, cognitive processing, autonomic and endocrine responses. It would be plausible to speculate that altered glutamatergic signaling in these principal cortico-limbic circuits may be reflected in diverse bipolar clinical symptomatology.

Glutamatergic findings in bipolar disorder are similar whether the patients are medicated or not. Of note, one of the studies demonstrated an inverse relationship between diurnal salivary cortisol levels and hippocampal glutamate concentration in bipolar patients. This finding reaffirms a critical link between neuroendocrine disturbance and glutamate transmission in bipolar disorder, implicating this key area in-

involved in memory, emotional regulation and stress response.⁷³ Overall, multiple, consistent and convergent evidence from genetic (not discussed here), postmortem, biochemical and imaging studies points to a principal role of glutamatergic dysregulation in the etiopathogenesis of bipolar disorder. Moreover, evidence links aberrant glial-neuron interactions and immuno-endocrine dysregulation with alterations in glutamatergic transmission.⁷⁴

ANTI-INFLAMMATORY TREATMENTS- THE INFLAMMATORY PATHWAY

Immune-inflammatory signaling involves an array of interacting cascading molecules. In brief, the long-chain omega-6 fatty acid arachidonic acid, derived from dietary linoleic acid via a series of transformation reactions by the enzymes desaturase and elongase, becomes acetylated and incorporated into membrane phospholipids. Phospholipid-bound arachidonic acid is mobilized via a calcium-dependant cytosolic isoform of phospholipase A2, and free arachidonic acid is a substrate for cyclooxygenase (COX)-mediated biosynthesis of prostaglandins (i.e. PGH₂), thromboxanes and prostacyclins, as well as lipoxygenase-mediated biosynthesis of leukotrienes. COX generated PGH₂ is converted to PGE₂ via PGE synthase and PGE₂ stimulates the biosynthesis of downstream pro-inflammatory cytokines including IL-6 at the level of transcription.⁷⁵ Pro-inflammatory cytokines including IL-6, IL-1 beta and TNF-alpha in turn stimulate hepatic biosynthesis of acute-phase proteins including CRP. In contrast to arachidonic acid, the long-chain omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are predominantly anti-inflammatory and EPA competes with arachidonic acid for metabolism by COX enzymes. In addition, COX and lipoxygenase metabolites of DHA and EPA (i.e. D- and E-series resolvins) have potent inflammation-resolving properties.⁷⁶

ANTI-INFLAMMATORY POTENTIAL OF LITHIUM AND VALPROATE

There is considerable preliminary evidence suggesting that traditional mood stabilizers modulate neuroinflammation. In two recent studies lithium was shown to have neuroprotective activity in preclinical models. In rat glial cells, pretreatment with lithium showed a significant anti-inflammatory potential, decreasing lipopolysaccharide-induced secretion of TNF-alpha, IL-1 beta, prostaglandin E₂ and nitric oxide.⁷⁷ Similarly, in an intracerebral hemorrhage model lithium reduced cell death, COX 2 expression, and reactive microglia in perihematomal regions in rats.⁷⁸ Interestingly, valproate

has also shown anti-inflammatory properties in preclinical models, modulating both systemic and CNS responses.⁷⁹ Nevertheless, not enough clinical evidence exists to support that these medications would exert neuroprotective effects in general, and specifically through immune and inflammatory pathways in particular.

RE-PURPOSED DRUGS WITH ANTI-INFLAMMATORY PROPERTIES

The adjunctive use of drugs with anti-inflammatory properties, such as omega-3 fatty acids, acetylsalicylic acid, celecoxib and minocycline is another area that has recently started to be explored. Omega-3 is nutritionally important fatty acids that include alpha-linolenic acid, DHA and EPA. A recent meta-analysis showed that EPA is a more effective component in the treatment of major depressive disorder than DHA.⁸⁰ As alluded to earlier, these molecules are supposed to compete for the biotransformation of eicosanoids such as prostaglandins and leukotrienes. In fact, competition for the biosynthesis of inflammatory mediators could be partially responsible for their anti-inflammatory effects. Although current evidence does not support the adjunctive use of omega-3 in the treatment of bipolar mania, some studies have demonstrated their efficacy in bipolar depression.⁸¹

The antibiotic and anti-inflammatory effects of minocycline inhibit apoptosis by attenuating microglial release of proinflammatory cytokines IL-1 beta, TNF-alpha and IL-6, while at the same time promoting the release of anti-inflammatory cytokine IL-10. However, the efficacy of minocycline has not been formally tested in mood disorders. Recently, a clinical trial with minocycline and aspirin was proposed and conducted but final results have not yet been published.⁸²

Acetylsalicylic acid irreversibly inhibits COX-1 and modifies the enzymatic activity of COX-2. COX-1 and COX-2 differentially modulate leukocyte recruitment during neuroinflammation. The clinical use of low dose aspirin has been primarily driven by its role as an antithrombotic and thrombolytic agent. Given the high rates of death from cardiovascular events in bipolar disorder, this action might be potentially advantageous in the management of bipolar disorder. Nevertheless, recent literature also supports the use of low dose aspirin in the management of mood disorders itself, more specifically to ameliorate depressive symptoms.⁸³ The COX-2 inhibitor celecoxib was tested in the treatment of depressive and mixed episodes in bipolar disorder in a short term randomized, controlled trial. That study showed some benefit of celecoxib in the treatment of depressive symptoms, but it remains unclear whether those benefits outweigh the risks at this point.⁸⁴

DEVELOPMENT OF BIOMARKERS- FOCUS ON CYTOKINES AND BDNF

Biomarker development is a multi-stage process which requires 1) proof of concept, 2) prospective validation in independent populations, 3) documentation of incremental information when added to standard risk markers, 4) assessment of effects on patient management and outcomes, and 5) cost-effectiveness.⁸⁵ Biomarkers have a variety of potential applications including diagnosis, monitoring of disease burden, prognosis, and prediction of treatment response. Sensitivity, specificity, positive and negative predictive value are statistical properties that are crucial to examine in order to determine the value of incorporating biomarkers into clinical practice. In psychiatry, such approaches appear premature as investigative efforts are currently being directed at acquiring evidence on the most relevant candidates. Nevertheless, the need for biomarkers is widely acknowledged by clinicians, researchers and patients alike. This need is arguably greatest for bipolar disorder, characterized by numerous presentations in terms of mood polarity and exceedingly high rates of comorbidity. The extant literature provides some clues in this respect, as an increasing number of studies point to abnormalities of components of the inflammatory pathway and neurotrophic signaling in different phases of the bipolar illness. This section of the paper attempts to summarize these findings with a focus on peripheral cytokines and brain derived neurotrophic factor measurements in the study populations.

PERIPHERAL CYTOKINES IN BIPOLAR DISORDER

In 2013, three meta-analyses incorporating somewhat different methodologies yielded generally convergent findings. In one report, Munkholm and colleagues examined 13 studies (using non-stimulated *in-vivo* studies only) published through January 2012 (n=556 BD, n=767 controls) and found that levels of TNF-alpha, soluble TNF receptor type 1 (sTNF-R1) and soluble interleukin-2 receptor (sIL-2R) were significantly elevated among manic BD patients vs. controls. In addition, sTNF-R1 and TNF-alpha were elevated among manic BD patients vs euthymic BD patients. Some studies demonstrated statistical trends towards increased levels of TNF-alpha and IL-6 among depressed BD patients vs. controls, and increased IL-6 in bipolar depression vs bipolar mania, however these findings were not significant in the meta-analysis. Only sTNF-R1 was significantly higher among euthymic BD patients vs controls.⁸⁶

In their subsequent meta-analysis, including articles up to January 2013, Munkholm and colleagues examined 18 stud-

ies (non-stimulated, *in vivo*; n=761 BD, n=919 controls). They examined all BD subjects together, including those who were manic, depressed, or euthymic and found that sIL-2R, TNF-alpha, sTNF-R1, sIL-6R and IL-4 (an anti-inflammatory cytokine) were higher among BD patients vs controls.³⁰

Finally, Modabbernia and colleagues examined 30 studies published through December 2012 (n=1351 BD, n=1248 controls). The larger number of studies is owing to the inclusion of studies using mitogen-stimulation, in addition to those examining only circulating levels of cytokines. They found that IL-4, IL-6, IL-10, sIL-2R, sIL-6R, IL-1RA (interleukin-1 receptor antagonist), TNF-alpha and sTNF-R1 were significantly elevated in manic BD patients vs controls. Non-significant trends were observed for IL-1beta and IL-6. Heterogeneity of findings was mitigated in subgroup analyses based on mitogen stimulation methodology. There were trends towards higher TNF-alpha and IL-10 levels among depressed BD patients vs controls. Among BD patients, IL-6 tended to be higher during mania than depression.⁸⁷

SERUM BDNF AS A BIOMARKER FOR BIPOLAR DISORDER

BDNF is important for neurogenesis, synaptic plasticity and dendritic growth. Much of the circulating BDNF originates from CNS neurons and glia. Preclinical models demonstrate high positive correlation between serum BDNF levels and brain BDNF expression.⁸⁸ Increasing BDNF expression in the hippocampus and prefrontal cortex produces antidepressant effects. The BDNF val66met polymorphism, associated with reduced function, may be implicated among children and adolescents with bipolar disorder and among adults with early onset BD.⁸⁹ Multiple studies have found reduced circulating BDNF protein levels during acute phases of bipolar disorder.⁹⁰ Studies have found negative associations between serum BDNF levels and manic and depressive symptoms, although there are also contradictory findings from small studies.⁹¹ Most studies include patients taking psychotropic medications, however convergent findings are reported among untreated participants.⁹² Greater reductions in BDNF are noted in bipolar disorder as compared to major depressive disorder.⁹³ A relatively recent systematic review and meta-regression analysis (n=548 BD, n=565 controls) reported reduced BDNF with large effect sizes (ES) for mania (ES -0.81) and bipolar depression (ES -0.97) versus controls. In contrast, differences in BDNF levels among euthymic BD patients versus controls were not significant and of modest magnitude (ES -0.20). There was substantial variability in findings in the euthymic phase, and both increasing age and longer illness duration were associated with reduced

peripheral BDNF levels and contributed to this variability.⁹⁴

One study, in which first episode bipolar patients in manic phase were examined, showed an increase in BDNF levels with treatment over time and episode remission.⁹⁵ Another study in which both manic and depressed patients received the same treatment i.e. extended release quetiapine showed an increase in BDNF with treatment in depressed subjects but a decrease in BDNF in manic/mixed episode patients.⁹⁶ In a study in which serum BDNF levels were investigated in manic patients before and after treatment, an increase in BDNF with lithium administration was seen.⁹⁷ In general, it can be concluded from the existing literature that BDNF levels are reduced in acute episodes and increase after treatment with mood stabilizing medications.

COMPARISON OF BIOMARKERS BETWEEN BIPOLAR DISORDER AND MAJOR DEPRESSIVE DISORDER

Increasing amounts of data suggest that inflammatory responses have an important role in the pathophysiology of depression. Most of the evidence that links inflammation and MDD comes from three observations:

- 1) MDD (even in the absence of medical illness) is associated with raised inflammatory markers.
- 2) Inflammatory medical illnesses-both CNS and peripheral-are associated with greater rates of major depression.
- 3) Patients treated with cytokines for various illnesses are at increased risk of developing major depressive illness.

Mean array value for inflammatory mediators/markers is higher in MDD than normal, non-depressed subjects. Approximately one-third of people with MDD have higher levels of inflammatory markers compared with normal, non-depressed population. These increases are more modest than in autoimmune or infectious disease-for example, 2-3 times higher than in healthy controls. However, small physiologic differences can have profound consequences over time, especially if they change in a consistent direction. Similar findings have been found in cardiovascular disease, stroke and diabetes.

Alterations in serum and CSF concentrations of a number of inflammatory markers, including cytokines, chemokines and acute phase reactant proteins, have been found in patients with MDD. The most replicated findings pertain to raised CRP and proinflammatory cytokines-TNF-alpha and IL-6-confirmed by at least two recent meta-analyses.^{98,99} A meta-analysis of 22 antidepressant treatment studies found that IL-1beta and IL-6 levels (but not TNF-alpha) decreased in response to therapy with SSRIs, along with a reduction in depressive symptoms.¹⁰⁰ These findings propose the possibility that inflammatory cytokines contribute to depressive symp-

toms, and that antidepressants block the effect of inflammatory cytokines in the brain.

The multiple mechanisms through which inflammation may act in precipitating the depression phenotype include:

- 1) Insensitivity to glucocorticoid inhibitory feedback
- 2) Reduced parasympathetic signaling
- 3) Reduced production of BDNF
- 4) Increased anterior cingulate cortex activity
- 5) Reduced hippocampal volume

This resonates with the concept of allostatic load. Allostasis has been described as the process of adaptation to acute stress, involving activation of both the sympathetic adrenomedullary and HPA axes in order to restore homeostasis when faced with a challenge. Allostatic load refers to the 'wear and tear' that the body experiences as a result of the activation of the above systems. It is not surprising that the progression of inflammation serves as a key mediator of the process of allostasis. A number of studies have shown a significant association

between allostatic load (measured using a composite score of inflammatory and metabolic markers) and medical health (eg, cardiovascular disease) and with mental ill health (such as major depression).

A summary of abnormalities of inflammatory biomarkers in major depressive disorder is given in Table 2.

p11 PROTEIN AND ITS RELATIONSHIP TO MOOD DISORDERS

The etiopathogenesis and treatment of major mood disorders have focused on monoaminergic (5HT, norepinephrine and dopamine) and amino acid (GABA, glutamate) receptors at plasma membrane. The time lag in the neuropsychiatric effects implicates intracellular neuroplasticity as primary in the mechanism of action of antidepressants and mood stabilizers. This review now discusses the function of a small acidic chaperone protein, p11 also known as S100A10 which inter-

Table 2. Abnormalities of inflammatory mediators in major depressive disorder

Study (year)	Type	Main findings
Dowlati et al. (2010) ⁹⁸⁾	Meta-analysis	Significantly higher concentrations of TNF-alpha and IL-6 in MDD patients compared to NC.
Hannested et al. (2011) ¹⁰⁰⁾	Meta-analysis	Serum levels of inflammatory cytokines—TNF-alpha, IL-1beta and IL-6 elevated in subjects with MDD. Levels of IL-1beta and IL-6 return to normal with response to antidepressant treatment but not TNF-alpha.
Liu et al. (2012) ⁹⁹⁾	Meta-analysis	The levels of sIL-2R, TNF-alpha and IL-6 significantly higher in MDD patients compared to NC. Sensitivity analysis found that European but not non-European subjects had higher levels difference of sIL-2R, TNF-alpha and IL-1beta between MDD and NC.
Young et al. (2014) ¹⁰¹⁾	Systematic review	Proinflammatory cytokines in both plasma and CSF known to influence the progression and severity of depressive disorders in different populations. Reviewed studies showed elevated serum levels of IL-1, IL-6, TNF-alpha, CRP and MCP-1 in MDD subjects. Mixed results with IL-8 serum levels and with IL-6 and MCP-1 CSF levels.
Bai et al. (2014) ¹⁰²⁾	Cross-sectional, case-control design	More than 2/3 of depressed patients complain of pain and somatic symptoms. Proinflammatory cytokines, including CRP, sIL-2R, sIL-6R, sP-selectin, MCP-1 and adiponectin were assessed by ELISA. Multiple regression analysis showed that sP-selectin was the only predictor for somatic and pain symptoms.
O'Donnovan et al. (2013) ¹⁰³⁾	Cross-sectional, case-control design	Patients with MDD who attempt or complete suicide have elevated inflammation compared to non-suicidal patients with MDD. Patients with MDD and high suicidal ideation had significantly higher inflammatory index scores (composite of TNF-alpha, IL-6, IL-10, CRP) compared to NC and MDD patients with lower suicidal ideation.

CRP: C-reactive protein, CSF: cerebrospinal fluid, ELISA: enzyme linked immune-sorbent assay, IL: interleukin, MCP-1: monocyte chemoattractantprotein-1, MDD: major depressive disorder, NC: normal control, TNF: tumor necrosis factor

acts with a number of serotonin receptors, in particular 5HT1B, 5HT1D and 5HT4 and has been implicated in mood regulation, nociception, ionic calcium uptake and cell polarization.

p11 is expressed in several brain regions implicated in the pathophysiology of mood disorders, including the nucleus accumbens, cerebral cortex and hippocampus.¹⁰⁴ Of clinical and translational relevance, p11 mRNA and protein are down-regulated in the anterior cingulate cortex and the ventral striatum from depressed individuals. Studies in human suicide victims have also found reductions of p11 mRNA in hippocampus and amygdala, indicating an important role of p11 in multiple brain regions associated with depression pathophysiology.¹⁰⁵ Conversely, antidepressants of several distinct categories-SSRIs, tricyclic antidepressants, monoamine oxidase inhibitors-as well as electroconvulsive therapy, increases p11 expression in frontal cortex and hippocampus of mice and rats. p11 knockout mice exhibit reduced neurogenic and behavioral responsiveness to SSRI administration. p11, by virtue of its ability to promote accumulation of 5HT receptors at the cell surface, serves as an amplification mechanism for 5HT receptor-mediated actions. p11 is expressed in peripheral blood cells and ongoing work should establish whether p11 peripheral expression can serve as a biomarker of depression and/or responsiveness to antidepressant treatment.

CONCLUSIONS

Bipolar disorder is different from other psychiatric conditions in the sense that whereas, most mental illnesses vacillate within a single register between symptom exacerbation and various degrees of recovery, flare ups in BD come in two distinct forms-mania and depression-and that often these episodes can take any of a nearly infinite number of combinations of mood disturbances. In the pathophysiology of bipolar disorder, consistent findings have emerged at a cellular level, providing evidence that BD is reliably associated with dysregulation of glial-neuronal interactions and with abnormalities more apparent in glial elements than in neurons. Among these glial elements are microglia-the brain's primary immune elements, which appear to be overactive in the context of bipolarity. Multiple studies now indicate that inflammation is also increased in the periphery of the body in both the depressive and manic phases of the illness, with at least some return to normality in the euthymic state. These findings are in line with changes in the HPA axis, such as reduced sensitivity to glucocorticoids which are known to drive inflammatory activation. The fact that bipolarity reaches so deeply into the core processes of life itself may enhance the understanding of why the disorder is so reliably associated with immune abnormalities and with a marked escalation in risk for the de-

velopment of multiple medical conditions that account for much of the increased mortality associated with the disorder.

As far as the development of reliable biomarkers for the disease is concerned, recent meta-analyses confirm that pro-inflammatory markers are increased and BDNF is decreased among adults with BD during acute episodes. Although constrained by a small number of studies with few subjects, there is also preliminary evidence that these markers change over time and in response to treatment. The extant literature strongly suggests that there is ample justification to continue research regarding these markers as potential clinical indicators in BD. For cytokines and BDNF to realize their full potential will require the consistent application of several methodological approaches. These include larger sample sizes, repeated measures and within-subject comparisons, incorporation of important covariates (age, sex, BMI, stressors) and sub-group analyses (BD subtype, family history and comorbidities). Preliminary data suggest that age and stage of illness may contribute to heterogeneity in findings. It is important for the field to consider the possibility that there are developmental and/or stage-related differences in the relationship of these markers to symptoms, course and treatment of BD. Validation of cytokines and BDNF would justify further investigation into whether explicitly attempting to modify levels of these markers, using anti-inflammatory and/or neurotrophin-based interventions, can yield benefits in terms of mood stabilization.

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