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Inflammatory cytokines and nuclear factor-kappa B activation in adolescents with bipolar and major depressive disorders

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ABSTRACT

Adults with bipolar disorder (BD) and major depressive disorder (MDD) have higher circulating levels of proinflammatory cytokines than healthy controls. However, it is not known whether pediatric-onset patients with BD or MDD show increases in levels of inflammation or activation of nuclear factor kappa B (NF-κB), a key transcription factor in inflammatory signaling. Circulating levels of inflammatory cytokines, as well as spontaneous and stimulated levels of activated NF-κB in total peripheral blood mononuclear cells, monocytes and lymphocytes were measured in adolescents with BD (n=18), MDD (n=13), or no psychiatric history (n=20). Participants had a range of mood symptoms at time of testing. Adolescents with BD had significantly higher spontaneous levels of NF-κB in peripheral blood mononuclear cells, monocyte and lymphocyte populations, and higher plasma levels of IL-1β than healthy controls. Following stimulation with recombinant human TNF-α, participants with BD and MDD both had greater increases in NF-κB in monocytes than controls. Further, greater stimulated increases of NF-κB in monocytes were associated with the current severity of depressive symptoms. The results are limited by the small sample and cross-sectional design. Interventions that target early immunological dysregulation should be examined in relation to long-term outcomes in youth with bipolar and depressive disorders.

Clinical Trial registration information: Early Intervention for Youth at Risk for Bipolar Disorder, https://clinicaltrials.gov/ct2/show/NCT01483391.

1. Introduction

Bipolar disorder (BD), one of the world's ten most disabling conditions, exerts a considerable toll on the psychological and physical health of the sufferer (Kupfer, 2005). The search for biological markers has yielded candidate endophenotypes including abnormal intracellular signaling cascades, inadequate cortical control over limbic activity when processing emotions, sleep and circadian rhythm dysregulation, heightened reward sensitivity, and low levels of brain-derived neurotrophic factor (Geddes and Miklowitz, 2013). Given the high rates of medical illness comorbidity in BD, the potential role of altered inflammatory activity as an illness mechanism has begun to receive attention (Goldstein et al., 2015a, 2015b).

It is well-established that inflammatory cytokines induce behaviors associated with depression, including changes in sleep, anhedonia, and decreased activity. As compared to healthy controls, adults with major depressive disorder (MDD) show increases in circulating levels of proinflammatory cytokines (Miller et al., 2009). Increases in systemic markers of inflammation are also found in patients with BD, especially among those in acutely depressed or manic states (Modabbernia et al., 2013; Brietzke et al., 2009; O'Brien et al., 2006; Guloksuz et al., 2010). These studies have focused almost exclusively on circulating levels of proinflammatory cytokines (e. g., IL-6) in adults with established MDD or BD. Fewer studies have examined upstream inflammatory signaling mechanisms such as nuclear factor kappa B (NF-κB), despite evidence that activation of these transcription factors plays a key role in the regulation of the inflammatory cascade and in responses to psychological stress (Keri et al., 2014; Pace et al., 2006; Slavich and Irwin, 2014; Wieck et al., 2013). Compared to healthy volunteers, higher levels of NF-κB activity have been found in

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adults with BD during depressed (Spiliotaki et al., 2006) and euthymic states (e.g., Amoruso et al., 2015). Barbosa et al. (2013) found a 7.2-fold increase in phosphorylated p65 NF-κB protein levels in euthymic bipolar I patients compared to healthy volunteers. These findings have been limited to the study of mixed peripheral blood mononuclear cell populations, despite evidence that activation of NF-κB in monocyte populations is key in initiating the inflammatory response in vivo.

Little is known about changes in the inflammatory biology of pediatric-onset major depression or BD. Limited cross-sectional data suggest that children with MDD show elevations in proinflammatory cytokines such as IL-1 beta (IL-1β) and IL-6 as compared to healthy controls (Henje Blom et al., 2012; Gabbay et al., 2009; Brambilla et al., 2004; Mitchell and Goldstein, 2014). No study has examined whether levels of the anti-inflammatory cytokine IL-10 are correspondingly lower in pediatric MDD or BD. Preliminary findings suggest increases in pro-inflammatory gene expression in adolescent offspring of adults with BD (Padmos et al., 2008) and increases in a systemic marker of inflammation, C-reactive protein (CRP) in adolescents with a bipolar spectrum disorder (i.e., bipolar disorder I,II, or not elsewhere classified) (Goldstein et al., 2011). In a within-group analysis of 123 bipolar adolescents and young adults (mean age 20.4), there was an association between high-sensitivity CRP levels (hs-CRP) and earlier age at illness onset, as well as the severity of depressive symptoms over 6 months (Goldstein et al., 2015a, 2015b). Importantly, mean levels of hs-CRP in this young sample were above the established threshold for increased risk for cardiovascular disease among adults.

The present study examined systemic and cellular markers of inflammation in adolescents who had lifetime BD spectrum disorders or MDD (currently in remission or with subsyndromal symptoms) compared to healthy controls. Our primary hypothesis was that adolescents with BD and MDD would show greater circulating concentrations of proinflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) and/or lower levels of IL-10, as compared to healthy volunteers. Secondarily, given the key role of the NF-κB transcription control pathway in regulating cellular expression of proinflammatory genes and increases in inflammatory cytokines, we hypothesized that among adolescents with BD or MDD, levels of NF-κB activation would be higher in peripheral blood mononuclear cells, including monocyte and lymphocyte subpopulations, than among adolescents with no psychiatric history. We measured levels of NF-κB signaling in spontaneous (unstimulated) states (i.e., activation in cells in the peripheral blood) to provide an index of in vivo activation of NF-κB. Additionally, we evaluated the ability of peripheral blood mononuclear cells and lymphocyte and monocyte populations to respond to a TNF-α challenge, because this proinflammatory cytokine induces a rapid (i.e., within 15 min) activation of the NF-κB signaling pathway, regardless of cell type, as would occur in an inflammatory response. NF-κB signaling was expressed as the change in intensity between unstimulated and stimulated cells. Finally, we examined the current severity of depressive and manic symptoms, body mass index, and medication regimens as additional independent variables or covariates for these group comparisons.

2. Material and methods

2.1. Participants

All subjects were between age 12 and 18 yrs. Patient participants had to meet lifetime DSM-IV-TR (American Psychiatric Association, 2000) criteria for bipolar disorder (BD), type I (n=7), type II (n=5), or not otherwise specified (n=6); or major depressive disorder (MDD, n=13). Bipolar disorder not otherwise specified was operationalized as per the Course and Outcome of Bipolar Youth criteria (Birmaher et al., 2009): (1) a distinct period of abnormally elevated, expansive, or irritable mood plus at least 2 DSM-IV-TR symptoms of mania (3, if irritable mood only) that caused a clear change in functioning, (2) mood and associated symptoms were present for ≥ 1 d, and (3) there have been at least 10 lifetime days in which the child met these mood, symptom, and functional change criteria. Youths who meet BD, not otherwise specified criteria are at substantially increased risk of converting to bipolar I or II disorder within 4 years (Birmaher et al., 2009).

Study candidates were recruited from consecutive admissions to a registry of patients who had completed an intake evaluation at the UCLA Child and Adolescent Mood Disorders clinic. Control subjects (n =20) had no lifetime history of mental health diagnoses. Seven healthy controls were recruited directly for this study through web advertising and flyers posted at the UCLA Medical Center. Data from 13 additional controls came from two prior UCLA studies of inflammatory functioning in healthy adolescents that used nearly identical screening, sample collection and assay procedures (i.e., multiplex cytokine assays, flow cytometry) as those in this study (Muscatell et al., 2015; Moieni et al., 2015). Potential participants were excluded if they had a current illness (cold, flu, infection) and/or were taking antibiotics, had preexisting inflammatory disease, met DSM-IV-TR criteria for current (prior month) alcohol or substance abuse or dependence disorder, or had pervasive developmental disorder. Participants (and if under 18, a parent) provided written informed consent for the study after receiving a full explanation of the procedures. The study was approved by the UCLA Medical Institutional Review Board.

2.2. Assessment

All adolescents with suspected mood disorders and the 7 healthy volunteers recruited directly for the study were interviewed by a research staff member using the Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Lifetime Version (KSADS-PL; Kaufman et al., 1997; Chambers et al., 1985). The mood disorder modules of the KSADS-PL were replaced by the KSADS Depression and Mania Rating Scales (Chambers et al., 1985; Axelson et al., 2003) to enable finer-grained distinctions between manic or hypomanic states varying in severity, duration and functional impairment. The interview covered symptoms experienced during the worst week of the past month (current ratings) and the worst 1–2 weeks lifetime (past ratings). Parents were interviewed separately and KSADS item consensus judgments – leading to axis I diagnoses - were made using all sources of information. The 13 healthy controls from the two prior UCLA studies had no psychiatric history as reported on the Structured Clinical Interview for DSM-IV Axis I disorders (First et al., 1995) interview.

The first study visit lasted 2–3 h and included dimensional ratings of current (prior week) manic/hypomanic symptoms using the Young Mania Rating Scale (YMRS; Young et al., 1978) and current (prior 2 weeks) depression symptoms using the Children's Depression Rating Scale-Revised (CDRS-R; Poznanski and Mokros, 1995). These clinical interview-based rating scales were not administered to control subjects who, by design and by diagnostic interview, had no history of a mood disorder or other axis I disorders (Table 1). Interrater reliability for the YMRS averaged. 98; for CDRS-R ratings, .93; for KSADS mania items, .90; and for KSADS depression items, .96 (intraclass rs).

Height and weight were obtained for all subjects, and Body Mass Index (BMI) was calculated (kg/m^2). Blood samples (non-fasting) were obtained between 9:00 a.m. and 1:30 p.m. There were no effects of time of day of blood sampling on levels of