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Increased cerebrospinal fluid interleukin-8 in bipolar disorder patients associated with lithium and antipsychotic treatment

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ABSTRACT

Inflammation has been linked to the pathophysiology of bipolar disorder based on studies of inflammation markers, such as cytokine concentrations, in plasma and serum samples from cases and controls. However, peripheral measurements of cytokines do not readily translate to immunological activity in the brain. The aim of the present study was to study brain immune and inflammatory activity. To this end, we analyzed cytokines in cerebrospinal fluid from 121 euthymic bipolar disorder patients and 71 age and sex matched control subjects. Concentrations of 11 different cytokines were determined using immunoassays. Cerebrospinal fluid IL-8 concentrations were significantly higher in patients as compared to controls. The other cytokines measured were only detectable in part of the sample. IL-8 concentrations were positively associated to lithium- and antipsychotic treatment. The findings might reflect immune aberrations in bipolar disorder, or be due to the effects of medication.

1. Introduction

Bipolar disorder (BD) is a severe psychiatric condition characterized by recurrent episodes of elevated (mania or hypomania), depressed, or mixed mood (Belmaker, 2004). Immune system dysregulation and inflammation have been suggested to play a role in the pathophysiology of BD (Goldstein et al., 2009). One line of research has focused on the role of cytokines, which have been shown to impact synaptic plasticity, neurotransmitter metabolism, and neurocircuits relevant to mood regulation (Haroon et al., 2012; McAfoose and Baune, 2009).

Several previous studies have investigated serum concentrations of cytokines and cytokine receptors in BD patients. The majority of these studies show altered cytokine serum concentrations during mood episodes, most commonly during mania (Goldstein et al., 2009; Munkholm et al., 2013a). A recent meta-analysis (Munkholm et al., 2013a) of 18 studies evaluating serum measurements of 17 cytokines, cytokine receptors, and receptor antagonists from both euthymic, manic and depressive bipolar patients found evidence of increased concentrations of the cytokines interleukin 4 (IL-4) and tumor necrosis factor alpha (TNFα), as well as the receptors soluble tumor necrosis factor receptor 1 (sTNF-R1), sIL-2R, and sIL-6R. Another meta-analysis investigating state related alterations showed increased sTNF-R1 concentrations in euthymic patients (Munkholm et al., 2013b). In a third meta-analysis of 30 studies (Modabbernia et al., 2013), evidence of increased concentrations of IL-4, IL-10, TNF-α, sTNF-RI, sIL-2R, sIL-6R, and IL-1RA in BD patients compared to control subjects was found.

However, due to the relative impermeability of the blood–CSF barrier, concentrations of cytokines and other proteins in serum or plasma differ from the concentrations in cerebrospinal fluid (CSF) (Bromander et al., 2012; Maier et al., 2005). Hence, peripheral measurements of cytokines do not necessarily reflect the immunological activity in the brain. Except for one small study from our group (30 patients and 30 controls) (Söderlund et al., 2011), all previous published studies have been restricted to serum and plasma analyses (Goldstein et al., 2009; Munkholm et al., 2013b).

Many previous studies have also been hampered by the lack of control for potential confounding influences on cytokine concentrations. For instance, in the meta-analysis referred to above (Munkholm et al., 2013b), only 38% of the studies reported on body

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mass index (BMI) or smoking status, factors that have been shown to influence plasma concentrations of cytokines (Esposito et al., 2003; Haack et al., 1999). Further, medication status needs to be accounted for in the analyses as mood stabilizers and antipsychotic drugs have been shown to potentially alter cytokine concentrations both *in vitro* and *in vivo* (Kim et al., 2007; Rapaport and Manji, 2001).

Here, our aim was to study brain immune and inflammatory activity in bipolar disorder. To this end, we compared CSF cytokine concentrations in a large group of euthymic BD patients with those of control subjects. We analyze the results in relation to clinical parameters including medication status.

2. Methods

2.1. Study population

The study included 121 BD patients and 71 control subjects. The work-up procedures for patients and selection of control subjects have been described in detail previously (Jakobsson et al., 2013; Ryden et al., 2009). Patients were recruited from the St. Göran Bipolar Project, enrolling patients from the Northern Stockholm psychiatric clinic, Stockholm, Sweden, between October 2005 and April 2008. Inclusion criteria for this study were an age of at least 18 years old and meeting the DSM-IV-TR criteria for BD spectrum disorder (bipolar disorder type 1, type 2, or not otherwise specified). To aid the diagnosis of BD, the Affective Disorder Evaluation (ADE) was used. This is a semi-structured interview that includes adapted versions of the mood and psychosis modules of the Structured Clinical Interview for DSM-IV, and was developed for the Systematic Treatment Enhancement Program of Bipolar Disorder (STEP-BD) project (Sachs et al., 2003). Co-morbid psychiatric disorders were screened for by utilizing the Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998). The ADE and M.I.N.I. interviews were conducted by board certified psychiatrists or residents in psychiatry. The final diagnostic decisions followed a best estimate procedure (Leckman et al., 1982; Roy et al., 1997) and were made by a consensus panel of experienced board-certified psychiatrists specialized in BD. To screen for alcohol and substance abuse, the self-report questionnaires the Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al., 1993) and the Drug Use Disorders Identification Test (DUDIT) (Berman et al., 2005) were used. The severity of BD was rated using the Clinical Global Impression (CGI) rating scales. The Montgomery-Åsberg Depression Rating Scale (MADRS) and the Young Mania Rating Scale (YMRS) were used to assess depressive and manic symptoms in patients. Euthymia was defined as MADRS <14 and YMRS <14.

The control subjects were 71 population-based controls, who were randomly selected by Statistics Sweden (SCB) and age- and sex-matched for a subset of patients. Control subjects underwent a psychiatric interview by an experienced clinician using the M.I.N.I. to exclude psychiatric disorders. Moreover, they completed the questionnaires AUDIT and DUDIT and underwent somatic examination, blood tests, and lumbar puncture. Exclusion criteria were overconsumption of alcohol (defined as elevated concentrations of carbohydrate deficient transferrin, >8 standard drinks per time more than two times per week, and/or amnesia and/or loss of control more than once per month), substance abuse, untreated endocrinological disorders, pregnancy, dementia, schizophrenia or bipolar disorder in first-degree relatives, neurological conditions other than mild migraines and coexistent psychiatric conditions other than past minor depressive episodes, isolated episodes of panic disorder, previous eating or obsessive compulsive disorder that had remitted spontaneously or with brief psychotherapy counseling.

The study was approved by the Regional Ethics Committee in Stockholm and carried out in accordance with the Declaration of Helsinki. All participants gave oral and written consent to participate in the study.

2.2. Cytokine measurements

Patients were in a stable euthymic mood as judged by a physician and by MADRS and YMRS scores <14 at the time of CSF sampling. CSF was obtained by lumbar puncture that occurred between 09.00 and 10.00 h following an overnight fast. A total volume of 12 ml of CSF was collected, inverted to avoid gradient effects and divided into aliquots immediately stored at −80 °C until analyzed. An identical procedure was performed for the control subjects. All samples in this study were thawed and refrozen once before analysis.

IL-6 concentrations were measured using a singleplex assay (Human IL-6 Ultra-sensitive kit) while the other cytokines (IL-1β, IL-2, IL-4, IL-5, IL-8/CXCL8, IL-10, IL-12, IL-13, TNF-α, and IFN-γ) were analyzed using the MSD 96-well multi-array and multi-spot human cytokine assay (Human Cytokine Assay Ultra-Sensitive kit), as described by the manufacturer (Meso Scale Discovery, Rockville, MD, USA). The detection limits were 0.61 pg/ml for all cytokines (0.61 is the lowest standard value in the assays and therefore this value was chosen as the detection limit). When a concentration under the detection limit was found, a value of half the detection limit was assumed. Inter- and intra-assay coefficients of variation were below 10% for IL-8. All samples were analyzed at the same occasion. Experienced and board-certified laboratory technicians who were blinded to clinical information performed all measurements.

To validate cytokine measurements with the Meso Scale ultra-sensitive kit, samples from 20 patients and 21 controls were reanalyzed using the Proseek Multiplex Inflammation I panel (Olink Bioscience, Uppsala, Sweden) (Assarson et al., 2014). Both serum and CSF was analyzed for these 41 subjects.

2.3. Analysis of CSF/serum albumin ratio

Serum and CSF concentrations of albumin were analyzed by immunonephelometry on a Beckman Immage Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA) at the Clinical Neurochemistry Laboratory in Mölndal, Sweden, using a method accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC). Intra- and inter-assay coefficients of variation were below 10%. The ratio between the albumin concentration in CSF (mg/L) and serum (g/L) was calculated and used to assess blood–CSF barrier function (Andersson et al., 1994).

2.4. Statistical analyses

Group differences were established by Mann–Whitney *U* test, Fisher's exact test, and/or ANCOVA (age and CSF/serum albumin ratio as covariates). Data not showing normal distribution (as tested by one-sample Kolmogorov–Smirnov test) were log 10-transformed prior to parametric tests. A multiple linear regression model was used to identify cytokine concentration predictor variables (using a stepwise procedure with criteria: probability-of-*F*-to-enter $\leqslant 0.050$, probability-of-*F*-to-remove $\geqslant 0.100$). Variables with a univariate correlation significance <0.25 were included in the regression analysis. Bonferroni correction was applied in pairwise comparisons to adjust for multiple comparisons. Level of significance was set at $p < 0.05$. SPSS Statistics version 21 (IBM Corp., Armonk, NY, USA) was used in all statistical analyses.