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Abnormal gene expression of proinflammatory cytokines and their receptors in the lymphocytes of bipolar patients

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Running head: Cytokines receptors in bipolar patients

Abstract

Objectives: Abnormalities of protein levels of proinflammatory cytokines and their soluble receptors have been reported in plasma of bipolar (BP) patients. In this study we tested the hypothesis that the mRNA expression of membrane-bound receptors for proinflammatory cytokines will be altered in the lymphocytes of BP patients.

Methods: We determined protein and mRNA expression of proinflammatory cytokines, and mRNA expression of their receptors in the lymphocytes from 29 drug-free, hospitalized BP patients and 30 drug-free normal control subjects. The subjects were diagnosed according to DSM-IV criteria. Plasma protein levels of cytokines were determined by ELISA; mRNA levels in lymphocytes were determined by the qPCR method.

Results: We found that mean mRNA levels of proinflammatory cytokines IL-1β, IL-6, TNF-α, their receptors, TNFR1, IL-1R1, and the antagonist IL-1RA were significantly higher in the lymphocytes of BP patients compared with normal controls.

Conclusions: This study suggests that the observed abnormalities of membrane-bound cytokine receptors may alter the functional response of cytokines in BP illness and that the mRNA levels of these receptors could be a potential biomarker.

Key words: biomarkers, proinflammatory cytokines, cytokine receptors, bipolar illness, lymphocytes, IL-1 receptors, IL-6 receptors, TNF-α receptors, gene expression, mRNA

Introduction

Abnormalities of the immune function in general, and abnormalities of cytokines in particular, are implicated in the pathophysiology of bipolar (BP) illness. The suggested abnormalities of the immune function in BP illness are based on both direct and indirect evidence. For example, the administration of cytokines, such as interferons (IFNs), to rats causes a syndrome known as “sickness behavior” that includes cognitive changes (1, 2). Also, the administration of IFNs to cancer patients induces sickness behavior (3), including cognitive changes that involve verbal memory, cognitive speed, and executive functions, features similar to BP illness and depressive illness. Although the symptoms of psychosis and mania have not been generally associated with IFN therapy, Du et al. (4) reported the development of manic symptoms with IFN-α therapy that improved after the cessation of IFN-α therapy, and Greenberg et al. (5) reported that treatment with IFNs caused the development of manic symptoms in melanoma patients.

That inflammatory processes are involved in BP illness is also based on the observation that proinflammatory cytokines are abnormal in the serum of patients with BP illness [for review and meta-analysis see Munkholm et al. (6, 7), Modabbernia et al. (8), and Hamdani et al. (9)].

For example, elevated levels of TNF-α have been reported in manic and depressive episodes (10-12). High levels of IL-6 and TNF-α have also been reported during mania, with IL-6 levels returning to the baseline after treatment with mood stabilizers (13, 14). Although the protein levels of proinflammatory cytokines and their soluble receptors have been studied in BP illness, the levels of their membrane-bound receptors, which are mediating the biological and functional effects of cytokines, have not been studied in BP patients. We hypothesized that specific membrane-bound receptor subtypes may be abnormally expressed in blood cells of BP patients.

We therefore determined the gene expression of the proinflammatory cytokines, IL-1β, IL-6, and TNF-α, and the cytokine receptors IL-1R1, IL-1R2, IL-1R antagonist (IL-1RA), IL-6R,

IL-6 signal transducer (IL-6ST), also known as glycoprotein 130 (Gp130), TNFR1, and TNFR2 in the lymphocytes of BP patients. Although the gene expression of IL-1β, IL-6, and TNF-α has been studied in the monocytes of BP patients (15), to our knowledge, gene expression of IL-1, IL-6, and TNF receptors has not been studied in BP patients or for that purpose in depressed patients.

Methods and Materials

Subjects

These studies were conducted in hospitalized patients with BP illness (18 to 65 years old) admitted to the Psychiatric Clinical Research Center, a part of the General Clinical Research Center, University of Illinois at Chicago. This study was approved by the Institutional Review Board of the University of Illinois at Chicago. All subjects gave informed consent for the study. The exclusion criteria were: a) any significant medical disease, such as renal, cardiovascular, pulmonary, gastrointestinal, neurological or rheumatologic; b) recent drug or alcohol abuse history; c) pregnancy; d) psychopathology, such as personality disorders, as identified by SCID administered at the baseline. Most of the patients were treated with mood stabilizing drugs prior to hospitalization. After admission to the research unit, the patients were kept drug-free up to two weeks before starting treatment. The mean washout period was 4.14 ± 2.91 days. Blood samples were drawn from the patients (n = 29), and the clinical assessments were performed at the end of drug-free period before the initiation of treatment.

The comparison subjects were non-hospitalized normal control (NC) subjects (n = 30) recruited for the study through advertisements in newspaper and/or on hospital notice boards, by referral from normal controls, or by referral from hospital employees. They were free of any psychiatric disorder or family history of major affective or schizophrenic illness. They were also free of major medical problems and were free of any medication for at least two weeks prior to

blood drawing. They abstained from alcoholic beverages for at least one day prior to testing. An informed consent was obtained from all NC subjects.

The patients and control groups were generally matched for age, sex, and race. Blood samples from BP patients and NC subjects were obtained in the morning under fasting condition.

Clinical Assessments

Patients were diagnosed as having BP illness according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria, derived by consensus between two trained raters and based on clinical interviews and other available clinical information. Diagnostic and clinical assessments were conducted at admission and at discharge. The discharge diagnosis was considered definitive. Symptom ratings included scores on the Hamilton Depression Rating Scale (HDRS) and the Clinician-Administered Rating Scale for Mania (CARS-M) (16). The BP group consisted of 25 BP manic and 4 BP depressed subjects.

Blood Processing

Thirty ml of venous blood was collected in tubes containing 3.8% (w/v) sodium citrate in DEPC treated water (1 vol: 9 vol blood) for plasma. The blood was centrifuged immediately at 210 *g* for 15 min. The platelet-rich plasma (PRP) was removed for platelet isolation. To the red blood cell (RBC) layer, 15 ml of saline was added, mixed gently, and then transferred on Ficoll (2:1 respectively). The sample was then centrifuged at 400 *g* for 40 min. The upper layer above the interface layer was removed and discarded. The interface layer was taken and processed for lymphocyte isolation. The isolated lymphocytes were stored at -80 ºC till assayed.

RNA Isolation

Total RNA was extracted, from lymphocytes, by resuspending the pellet in TRIZOL reagent (Invitrogen) according to the manufacturer's instructions and treated with DNAse 1 (Invitrogen, USA). The RNA yield was determined by absorbance at 260 nm using

NanoDrop®ND-1000 (NanoDrop Technologies, Montchanin, DE, USA). RNA quality was assessed using Agilent Bioanalyzer 2100 (Agilent). All samples had 28S/18S ratios >1.2 and RNA integrity number (RIN) above 6.6. The mean RIN was 8.1 ± 0.7.

mRNA Determination

Expression levels of mRNA were determined using a two-step real-time RT-PCR (qPCR) method. One ug of total RNA was reverse transcribed using 50ng random hexamers, 2mM dNTP mix, 10 units ribonuclease inhibitor, 50 mM Tris–HCl (pH 8·3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, and 200 units MMLV-reverse transcriptase (Invitrogen) in a final reaction volume of 20 µl. Reverse transcription was performed at 37°C for 60 min, and enzymes were denatured at 70°C for 15 minutes. The cDNA was stored at -20°C.

Real-time PCR was performed with a MX3005p sequence detection system (Agilent) using pre-designed Taqman gene expression assays (Applied Biosystems, Grand Island, NY, USA) description is given in Table 1. The stability and optimal number of housekeeping genes was determined using geNORM version 3.4 (PrimerDesign Ltd, Southamptom, UK) according to the manufacturer's instructions (17). This comparison identified ACTB and GAPDH as the most stable housekeeping genes. PCR efficiency for all genes, after 5-log dilution series of pooled cDNA, was similar. For each primer/probe set, qPCR reaction was carried out using 10 µl of cDNA (diluted 1:10) in 1X TaqMan Universal PCR Master Mix (Applied Biosystems, Grand Island, NY, USA) per the manufacturer's instructions. Each qPCR plate included a "no reverse transcriptase" and "no template" control to eliminate non-specific amplification and each sample was assayed in triplicate.

For qPCR gene expression analysis, raw expression data (C_t) were normalized to the geometric mean of the two housekeeping genes. Outliers were excluded if the normalized (delta C_t) values were greater than two standard deviations from the group mean. Relative expression

levels, reported as fold change, were determined by the $2^{-(\Delta\Delta Ct)}$ method, where ΔΔCT = (CT target - CT normalizer) subject - (CT target - CT endogenous gene) control (Applied Biosystems User Bulletin No. 2). ΔCT values are used for further statistical analysis.

Determination of Plasma Protein Levels Using ELISA

Levels of proinflammatory cytokines were determined in plasma aliquots (100 µL) by enzyme-linked immunosorbent assay (ELISA) using commercially available Quantakine® kits (R & D Systems, Inc., Minneapolis, MN) for human IL-1ß, human IL-6, and human TNF-α, according to the manufacturer's instructions.

Statistical Analysis and Effect of Confounding Variables

We analyzed the data using SAS 9.2 statistical software package. First we used two sample t-test to compare NC subjects with BP patients. In order to examine the effect of confounding variables, we used generalized linear model (PROC GLM in SAS) for each outcome measure to compare those two groups adjusting for fixed covariates like age, sex and race. To examine the association between group and gender we performed a contingency chi-square test. Pearson correlation matrix was used to determine the relationship between the symptom rating scores and the cytokine mRNA and protein measures.

Results

The demographic and clinical characteristics of BP patients and NC subjects are summarized in Table 2.

mRNA Expression Levels of Proinflammatory Cytokines in the Lymphocytes of BP Patients and NC Subjects

We determined the mRNA levels of the proinflammatory cytokines, IL-1β, IL-6, and TNF-α in the lymphocytes obtained from 29 BP patients and 30 NC subjects. The mean mRNA

expression levels in the BP and NC subjects are shown in Figure 1 and in Table 3. When we compared the gene expression levels of the proinflammatory cytokines at the baseline period between the BP patients and the NC subjects, we found that the mRNA levels of IL-1β were significantly increased in BP patients compared with NC subjects (Fig. 1, Table 3). The gene expression levels of IL-6 were also significantly increased in the lymphocytes of BP patients compared with NC subjects (Fig. 1; Table 3). Similarly, the mRNA expression of TNF-α was significantly increased in the BP patients compared with NC subjects, as shown in Figure 1 and Table 3. These results thus indicate significant increases in the mRNA levels of proinflammatory cytokines in BP subjects compared with the NC group.

mRNA Expression Levels of Cytokine Receptors in the Lymphocytes of BP Patients and NC Subjects

Since we observed an increase in the mRNA levels of proinflammatory cytokines in the lymphocytes of BP patients, we then examined if the mRNA expression of their receptors was also altered in the lymphocytes of these patients. We therefore determined the mRNA expression of IL-1R1, IL-1R2, IL-1RA, IL-6R, Gp130, TNFR1, and TNFR2 in the lymphocytes of BP patients and NC subjects.

We observed that the mRNA expression of IL-1R1 was significantly higher, mRNA expression of IL-1R2 was not significantly different, and mRNA expression of IL-1RA was also significantly increased in the lymphocytes of BP patients compared with NC subjects (Fig. 2; Table 3).

The mRNA expression of TNFR1 was significantly higher in the lymphocytes of BP patients compared with NC subjects, but mRNA expression of TNFR2 was not significantly different from that of NC subjects (Fig. 2; Table 3). Thus, these results show increased mRNA expression of IL-1R1, IL-1RA, and TNFR1, and no change in IL-1R2, and TNFR2 in BP patients compared with NC subjects.

We also determined the gene expression of IL-6R and IL-6ST (also known as Gp130). We did not find any significant difference in the gene expression of IL-6R and Gp130 between BP patients and normal controls, as shown in Figure 2 and Table 3.

Protein Expression Levels of the Proinflammatory Cytokines in the Plasma of BP Patients and NC Subjects

We determined the protein expression levels of the proinflammatory cytokines, IL-1β, IL-6, and TNF-α, in the plasma obtained from NC subjects and BP patients. The mean protein expression levels of IL-1β, IL-6, and TNF-α are shown in Figure 3. We observed that the protein expression levels of IL-1β were significantly higher in the plasma of BP patients compared with NC subjects (Fig. 3, Table 4). Similarly, we found that the protein expression levels of IL-6 and TNF-α were significantly higher in the plasma of BP patients compared with NC subjects (Fig. 3; Table 4), but to a smaller extent.

Effect of Confounding Variables and the Relationship to the Severity of Illness

To examine the effect of confounding variables we used generalized linear model (PROC GLM in SAS) for each outcome measures to compare those two groups adjusting for fixed covariates like age, sex and race. Age was found to be non-significant for all outcomes. Gender was found significant for IL-1R1 mRNA measure, and race was significant for IL-1β protein measure. Overall results for GLM approach matched with the t-test results. To examine the association between group and gender we performed a contingency chi-square test and found no significant association.

In order to examine if any of the cytokine measures were related to the severity of illness, we examined the correlation between the cytokine measures and the CARS-M scores in BP patients determined at the baseline. We found a highly significant positive correlation between IL-6 protein levels and CARS-M scores ($r = .492$, $p=.03$), but not between other

cytokines measures, suggesting that IL-6 plasma protein levels could be used as an index of the severity of illness in BP patients.

Discussion

In this study we found that the mRNA expression levels of the proinflammatory cytokines (IL-1β, IL-6, and TNF-α) were significantly higher in the lymphocytes of BP patients compared with NC subjects. We also found that the mRNA expression levels of IL-1R1, IL-1RA, and TNFR1 were significantly higher in the lymphocytes of BP patients compared with NC subjects. No significant differences in the mRNA expression levels of IL-1R2, IL-6R, Gp130, and TNFR2 were observed in the lymphocytes of BP patients compared with NC subjects. Our results also indicate that this increase in the mRNA levels of proinflammatory cytokines was associated with increased protein expression levels of the cytokines, IL-1β, IL-6, and TNF-α in the plasma of BP patients. However, while we found a large increase in the gene expression levels of these cytokines in the lymphocytes of BP patients, the magnitude of the increase in protein expression of cytokines in the plasma, although significant, was not as high as that of the mRNA expression.

Although the protein expression levels of the proinflammatory cytokines and their soluble receptors in the plasma/serum of BP patients have been studied by several investigators (18, 19), most of whom found increased levels of proinflammatory cytokines in the serum of BP patients [see review and meta-analysis (6-8)], the gene expression levels of IL-1β, IL-6, and TNFα in the white cells of BP patients have only recently been reported by Padmos et al. (15), who found increased mRNA levels of the proinflammatory cytokines IL-1β, IL-6, and TNF-α in the monocytes of BP patients. Our results of increased mRNA levels of IL-1β, IL-6, and TNF-α in lymphocytes of BP patients appear to be similar to those reported by Padmos et al. (15).

Proinflammatory cytokines (IL-1, IL-6, TNF-α) exert several biological effects on a wide variety of target cells through specific plasma membrane receptors (20). Thus, for IL-1, two

types of receptors have been cloned, known as IL-1R1 and IL-1R2 (21, 22), of which only IL-1R1 initiates signal transduction while IL-1R2 presumably functions solely as a ligand sink or as a decoy receptor (18, 19). Thus, most of the IL-1 signal has been shown to be transmitted through IL-1R1 (23).

Receptor antagonists bind specifically to a cytokine receptor but are devoid of biologic activity (19). These antagonists compete with true biologically active cytokines for binding to the same membrane receptors. The only example of this type of antagonist is the human IL-1 receptor antagonist (IL-1RA) described by Hannum et al. (24). The inhibitory effect of IL-1RA lies in its ability to bind to the IL-1 receptor and inhibit the binding and subsequent signaling of both forms of IL-1, i.e., IL-1α and IL-1β (25). To examine if increased mRNA expression of IL-1β in BP patients is associated with its altered biological responses, we determined the gene expression of its membrane receptors and its antagonist. We found that while there was a significant increase in the gene expression of IL-1R1 and IL-1RA, however, the gene expression of IL-1R2 was not significantly different in the lymphocytes of BP patients compared with NC subjects.

The biological effects of IL-6 on its target cells are mediated through its receptor complex, IL-6R, and the signal transducing protein Gp130 (26, 27). The signaling is initiated by binding of IL-6 to IL-6R forming an IL-6/IL-6R heterodimer that then associates with Gp130, leading to the activation of various signaling pathways. We therefore determined the gene expression of both IL-6R and Gp130 to examine if increased exposure to IL-6 is associated with an abnormality of signal transduction. When we compared the gene expression of IL-6R and Gp130 in the lymphocytes of BP patients and NC subjects, we did not find a significant difference either in IL-6R or in Gp130 between these two groups.

Among the receptors for these proinflammatory cytokines, the TNFR1is probably quite important in BP illness since its activation by TNF-α results in apoptosis (28). TNF-α produces its intracellular signals by binding with high affinity to two distinct cell surface receptors, known

as TNFR1 and TNFR2. Although these two receptors exist also in soluble forms, only membrane-bound TNF receptors function as transducing elements able to produce intracellular molecular signals. Signal transduction pathways activated by TNFR1 and TNFR2 are diverse and include G protein-mediated activation of protein kinase A (PKA), phospholipase C (PLC), and phospholipase A2 (29-31). Although both TNFR1 and TNFR2 cause activation of transcription factors, such as nuclear factor kappa beta (NF-kB), only TNFR1 can activate the caspase pathway leading to apoptosis (28). Thus, TNFR1 initiates the majority of TNF-α biological activities (32). We found that the gene expression of only TNFR1, and not TNFR2, was significantly increased in the lymphocytes of BP patients compared with NC subjects.

Whereas soluble cytokine receptors for IL-1, IL-6, and TNF-α have been studied in the plasma of BP and depressed patients, to our knowledge, the membrane-bound receptors for these three proinflammatory cytokines have not been studied in either BP or depressed patients. The soluble receptors for these cytokines, which have been studied in the plasma of BP patients, may arise from the proteolytic cleavage of the extracellular domain of the original membrane-bound receptors or by synthesis from alternatively spliced variants (19, 33). They circulate at high concentrations in plasma, can induce cell activation, act as chaperones to extend cytokine bioavailability, or inhibit cytokine signaling (18, 33). However, the biological effects of the cytokines are mediated through their interactions with the membrane-bound receptors through several signaling mechanisms, as stated before. While the soluble receptors can compete with cytokines, such as TNF-α, they are not able to participate in the signaling mechanisms.

Abnormalities of proinflammatory cytokines have been reported in the plasma of BP patients [for review and meta-analysis see (6-8)]. Increased IL-1β plasma protein levels in BP patients have been reported by Drexhage et al. (34) and Padmos et al. (15), but Ortiz-Dominguez et al. (35) found no change. Soluble IL-1R (sIL-1R) was found to be increased by Hope and colleagues (36-38), but not by Tsai et al. (39). Increased TNF-α in plasma of BP

patients is reported by Kapczinski et al. (40, 41) and O'Brien et al., (12, 42), but no difference was found by Barbosa et al. (10) and Ortiz-Dominguez et al. (35). Most reports find increased levels of sTNFR1 in BP patients (10, 34, 36) but not of TNFR2 (10). Increased IL-6 levels were reported by Maes et al. (11), Brietzke et al. (13), but not by Ortiz-Dominguez et al. (35) or by Kapczinski et al. (40). No differences in IL-1RA were observed between BP patients and normal controls (36). In summary, the meta-analysis by Munkholm and colleagues (6, 7) concluded that the levels of TNF-α, the sTNFR1, and the sIL-2R were elevated in BP manic patients compared with controls. It is difficult to compare our results of membrane-bound receptors for IL-1, IL-6, or TNF-α with soluble receptors since these have different functions.

Our results of the receptors' gene expression appear to be very interesting. As mentioned earlier, the IL-1R1 and TNFR1 appear to play a significant role in signal transduction and these are the two receptors that appear to be abnormally expressed in BP patients, suggesting an abnormal biological function of IL-1β and TNF-α in BP illness. Our observation of increased IL-1RA in BP patients may be important, suggesting abnormalities of IL-1β function in BP illness.

Thus, there are several consistent reports, including our study, of increased levels of proinflammatory cytokines in the serum of BP patients compared with NC subjects [see meta-analysis (6-8)]. However, the reasons and the source of this increase in the proinflammatory cytokines in the serum are not clear. Cytokines are synthesized in the periphery primarily by white cells (monocytes and lymphocytes) (14, 43) and there is evidence to suggest that the white cells are activated in BP illness (34), thereby suggesting that the increase of cytokines in the serum of these patients may be related, at least in part, to the activation of the white cells, or to an increased number of white cells. Cytokines are also shown to be produced in the brain (44, 45), primarily by macrophages and possibly also by neurons. Bidirectional movement of cytokines between the periphery and the CNS has been suggested (46-48). Although the cytokines in general cannot cross the blood-brain barrier (BBB) (48), they may do so under

certain conditions. The three pathways by which cytokines may move in either direction have been suggested: (i) cytokines may move across the BBB in certain areas where the barrier is weak; (ii) cytokines may be transferred across the BBB by carriers or transporters; and (iii) increased levels of cytokines in these patients may make the BBB weak in certain areas (46-48). As stated earlier, the cytokines are also produced in the brain. The observation that cytokines' gene expression is increased in the lymphocytes of BP patients may suggest that increased levels of cytokines in this disorder are primarily related to the peripheral increase in cytokines. However, increased levels of cytokines have been reported in the brain of BP (49) and suicidal subjects (50). One of the reasons for this increase in the brain of these subjects may be related to their transport from the periphery where their levels are increased. It is also possible that increased levels of gene expression both in the periphery and in the brain may cause increased levels of cytokines in both serum and in the brain, as has been observed by us in the case of suicidal patients (50).

Whether the increase in the cytokine levels in the brain and periphery is related to their mRNA increase respectively, or also related to the bidirectional movement of cytokines is not clear; but it does indicate a relationship between the increased gene expression in the white blood cells and increased gene expression in the brain. Thus, peripheral cytokines may mirror their concentrations in the brain. Our studies suggest that white blood cells (lymphocytes and monocytes) are a useful tissue for studying mRNA levels and that the gene expression of proinflammatory cytokines could be a potential biomarker for BP illness or other mental disorders.

Limitations

Our studies have some limitations. We do not have available data on body mass index (BMI) or on smoking history of the subjects and hence the effect of these variables on the cytokines and their receptors could not be ascertained. Also, although the samples size is

adequate it is not large. This is primarily because we studied patients admitted to the research ward and not the outpatients.

Conclusion and Future Studies

In summary, our studies of gene expression of the membrane-bound receptors for IL-1β, IL-6 and TNF-α are quite significant. Although there are several studies of soluble receptors of cytokines in BP illness, to our knowledge, the expression of their membrane-bound receptors has not been studied in BP patients. Whereas studies of proinflammatory cytokine levels and their soluble receptors are important in understanding the role of these inflammatory measures in BP illness, the study of membrane-bound receptors for their regulation is important because the functional and biological effects of the cytokines are ultimately determined by the sensitivity of the membrane-bound receptors. The studies of the receptors are thus the initial step examining the main component of the cytokine function. It will be further important to study the downstream signaling cascade to evaluate the eventual functional effects of the cytokines. The important components of the signaling cascade of the receptors which need to be studied are thus, MyD-88, NFK-β and Caspase 8. The study of these intermediate components and transcription factors will provide better understanding of the role of cytokines in the pathophysiology of BP illness.

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Table 1. TaqMan primers/probes used for qPCR analysis

	TaqMan accession	Probe location (exon boundry)	Assay function
ACTB	Hs99999903_m1	1-1	House Keeping (HK)
GAPDH	Hs99999905_m1	3-3	HK
IL-1ß	Hs01555410_m1	3-4	target gene
IL-1RN (IL-1RA)	Hs00893626_m1	4-5	target gene
IL-1R1	Hs00991010_m1	7-8	target gene
IL-1R2	Hs00174759_m1	6-7	target gene
IL-6	Hs00985639_m1	2-3	target gene
IL-6R	Hs01075666_m1	5-6	target gene
IL-6ST (Gp130)	Hs00174360_m1	13-14	target gene
TNF-α	Hs99999043_m1	1-2	target gene
TNFRSF1A	Hs00533560_m1	1-2	target gene
TNFRSF1B	Hs00961755_m1	9-10	target gene

Table 2. Demographic Characteristics of Bipolar Patients and Normal Control Subjects

Group	Age (Years)	Gender (M/F)	Race	CARS-M
Normal Controls (n = 30)	34.6 ± 13.5	17 M / 13 F	5 Asian 7 Black 2 Hispanic 16 White	--
Bipolar Patients (n = 29)	31.6 ± 11.9	18 M / 11 F	1 Asian 8 Black 2 Hispanic 18 White	19.3 ± 12.6

Values are the mean ± SD.
Abbreviations: CARS-M, Clinician-Administered Rating Scale for Mania; F, female; M, male

Table 3. Mean mRNA Expression Levels of Proinflammatory Cytokines and Their Membrane-bound Receptors in the Lymphocytes of Bipolar Patients and Normal Control Subjects

Variable	Normal Controls (n = 30) Mean ΔCT ± SD	Bipolar Patients (n = 29) Mean ΔCT ± SD	t	p
TNF-α	9.06 ± .51	7.12 ± .53	- 14.24	<.0001
IL-1β	7.79 ± .48	6.76 ± .27	- 10.12	<.0001
IL-6	13.65 ± .50	12.42 ± .53	- 8.70	<.0001
TNFR1	4.89 ± .48	4.41 ± .51	- 3.67	<.0005
TNFR2	4.77 ± .32	4.58 ± .63	- 1.40	.17
IL-1R1	7.48 ± .54	7.12 ± .53	- 2.62	.01
IL-1R2	5.76 ± .47	5.93 ± .45	- 1.22	.23
IL-1RA	3.05 ± .37	2.78 ± .49	- 2.29	.02
IL-6ST (Gp130)	7.32 ± .53	7.40 ± .60	.24	.81
IL-6R	6.40 ± .53	6.23 ± .43	-1.21	.23

Table 4. Mean Protein Expression Levels of Proinflammatory Cytokines and Their Membrane-bound Receptors in the Plasma of Bipolar Patients and Normal Control Subjects

Variable	Normal Controls (n = 30) Mean (pg/ml) ± SD	Bipolar Patients (n = 29) Mean (pg/ml) ± SD	t	p
TNF-α	1.46 ± .39	1.85 ± .99	2.41	.02
IL-1β	1.26 ± .81	2.05 ± 1.26	4.35	<.0001
IL-6	.99 ± .39	1.29 ± .70	2.04	.04

Figure Legends

Figure 1. Mean mRNA expression levels of proinflammatory cytokines, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in the lymphocytes of BP patients and NC subjects. The data are shown as fold change in mRNA levels. Values are fold change ± S.E.M.

*p< .05

Figure 2. Mean mRNA expression levels of receptors for proinflammatory cytokines, IL-1R1, IL-1R2, IL-1RA, IL-6R, Gp130, TNFR1, and TNFR2 in the lymphocytes of BP patients and NC subjects. The data are shown as fold change in mRNA levels. Values are fold change ± S.E.M.

*p< .05

Figure 3. Mean protein expression levels of IL-1β, IL-6, and TNF-α in the plasma of BP patients and NC subjects. Values are mean ± SD.

*p < .05

Figure 1.

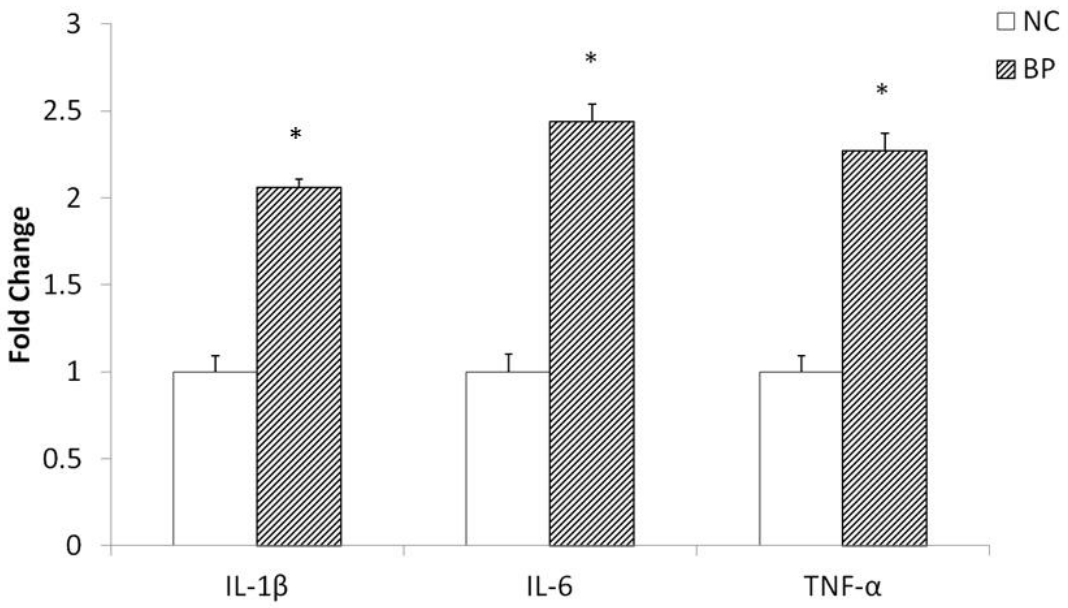


Figure 2.

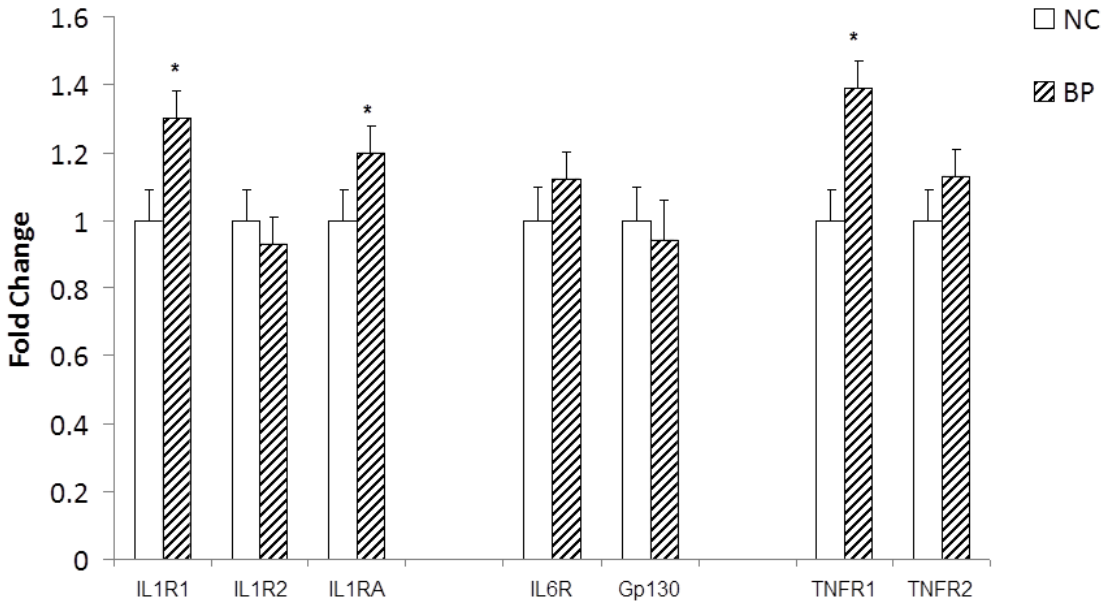
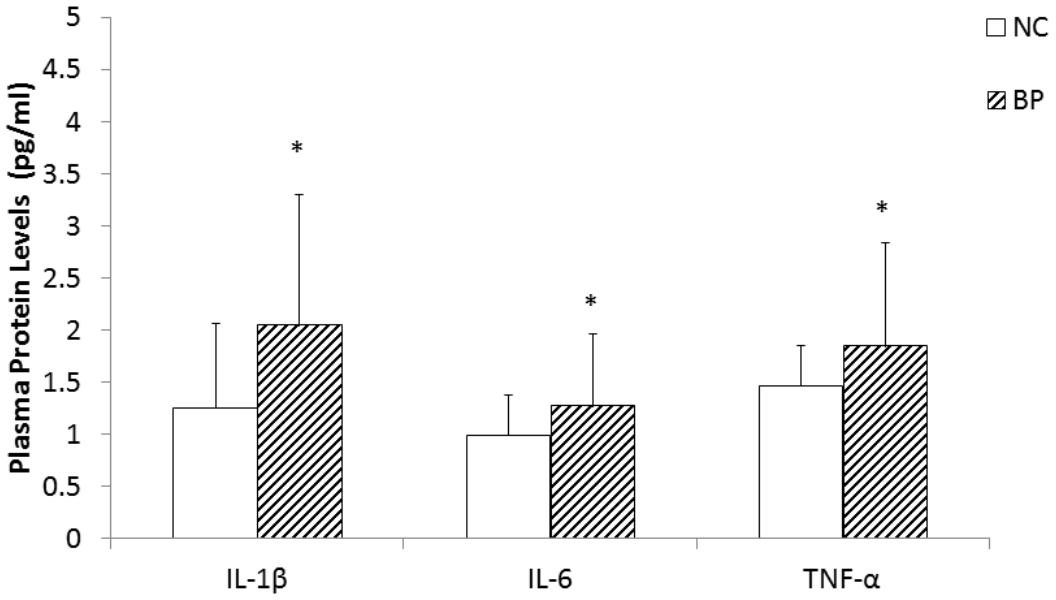

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Fold Change
*
*
*
IL1R1
IL1R2
IL1RA
IL6R
Gp130
TNFR1
TNFR2
NC
BP

Figure 3.


Plasma Protein Levels (pg/ml)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
NC
BP
*
*
*
IL-1β
IL-6
TNF-α