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Abnormal gene expression of proinflammatory cytokines and their membrane-bound receptors in the lymphocytes of depressed patients

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Abstract

Abnormalities of protein levels of proinflammatory cytokines and their soluble receptors have been reported in plasma of depressed patients. In this study, we examined the role of cytokines and their membrane-bound receptors in major depressive disorder (MDD). We determined the protein and mRNA expression of proinflammatory cytokines, interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α , and mRNA expression of their membrane-bound receptors in the lymphocytes from 31 hospitalized MDD patients and 30 non-hospitalized normal control (NC) subjects. The subjects were diagnosed according to DSM-IV criteria. Protein levels of cytokines were determined by ELISA, and mRNA levels in lymphocytes were determined by the qPCR method. We found that the mean mRNA levels of the proinflammatory cytokines IL-1 β , IL-6, TNF- α , their receptors, TNFR1, TNFR2, IL-1R1 and the antagonist IL-1RA were significantly increased in the lymphocytes of MDD patients compared with NC. No significant differences in the lymphocyte mRNA levels of IL-1R2, IL-6R, and Gp130 were observed between MDD patients and NC. These studies suggest abnormal gene expression of these cytokines and their membrane-bound receptors in the lymphocytes of MDD patients, and that their mRNA expression levels in the lymphocytes could be a useful biomarker for depression.

Keywords

major depressive disorder; IL-1 β ; IL-6; TNF- α

1. Introduction

There is some evidence to suggest that neuroimmune abnormalities may be associated with depression Leonard and Myint (2009). There are many interactions of the neuroimmune and neuroendocrine systems and since abnormalities of the neuroendocrine system have been

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Conflict of interest

All authors declare that they have no financial interests or potential conflicts of interest related directly or indirectly to this work.

observed in depressive illness this has led to the suggestion that abnormalities of the immune system may also be involved in brain disorders such as depression. Among the major mediators of neuroimmune function are the proinflammatory cytokines which are released from the immune cells. Recently several investigators have proposed a cytokine hypothesis of depression (Miller et al., 2009; Schiepers et al., 2005).

Cytokines, generally known as chemical messengers between immune cells, comprise a heterogeneous group of messenger molecules produced by immunocompetent cells, such as lymphocytes and macrophages. They regulate the immune responses and interact with the central nervous system (CNS). There is some evidence to suggest the involvement of cytokines in depression. Several studies report that the administration of cytokines to animals or humans causes behavior known as sickness behavior which is similar to depression (Capuron et al., 2004; Dantzer, 2001a, b; Dantzer and Kelley, 2007). In humans, it has been reported that the administration of proinflammatory cytokines, such as interferon (IFN)- α or IFN- γ to cancer patients causes symptoms similar to depression (Capuron et al., 2001; Capuron et al., 2004; Dantzer et al., 1999). All these evidences taken together suggest the involvement of proinflammatory cytokines in the etiology of depressive illness.

However, the main direct evidence suggesting the involvement of cytokines in depression is derived from the observation of several studies showing that the levels of proinflammatory cytokines and their soluble receptors are abnormal in the plasma of depressed patients[see reviews by Dowlati et al. (2010), Hiles et al. (2012), Howren et al.(2009), Liu et al. (2012), Schiepers et al. (2005)]. A meta-analyses by Dowlati et al.,(2010) and by Hiles et al. (2012) indicated that the levels of proinflammatory cytokines IL-1 β , IL-6, and TNF- α , as well as the IL-1 receptor antagonist (IL-1RA) are increased in patients with depressive illness. It has recently been reported by Cattaneo et al. (2013) that the leukocyte mRNA levels of IL-1 β , IL-6, and TNF- α were significantly higher in depressed patients compared with normal controls.

Like the neurotransmitter receptors, the biological effects of cytokines are mediated through their membrane-bound receptors (Hohmann et al., 1989). Thus proinflammatory cytokines exert their biological effects on a wide variety of target cells through the specific plasma membrane-bound receptors. Cytokine receptors exist in two forms: (i) soluble cytokine receptors, and (ii) membrane-bound cytokine receptors (Fernandez-Botran, 1991). The soluble cytokine receptors arise from the proteolytic cleavage of the extracellular domains of the membrane-bound receptors or by synthesis from alternatively spliced variants. Thus, whereas the membrane-bound receptors are involved in the signal transduction system mediating the biological effects of cytokines, soluble receptors are devoid of such effects.

Whereas protein and gene expression of these cytokines and their soluble receptors have been studied in depression (Cattaneo et al., 2013; Dowlati et al., 2010; Schiepers et al., 2005), to our knowledge the gene expression of membrane-bound cytokine receptors have not been studied in the blood of patients with depression. The gene expression studies may possibly be more appropriate biomarkers for depressive illness, as has recently been suggested by studies of Padmos et al. (2008) for bipolar (BP) illness, who determined the

gene expression of proinflammatory cytokines IL-1 β , IL-6, and TNF- α as well as their protein expression in monocytes of BP patients.

In light of these observations, we determined the gene and protein expression of the proinflammatory cytokines IL-1 β , IL-6, TNF- α , as well as the gene expression of their receptor subtypes, such as IL-1R1, IL-1R2, TNFR1, TNFR2, IL-6R1 and IL-6 signal transducer (IL-6ST), also known as glycoprotein 130 (Gp130), and the receptor antagonist for IL-1, known as IL-1RA, in the lymphocytes obtained from drug-free, hospitalized depressed patients and normal control subjects.

2. Materials and methods

2.1 Subjects

The subjects for this study were hospitalized patients with major depressive disorder (MDD) and non-hospitalized NC subjects. The patients were admitted to the research wards of the University of Illinois General Clinical Research Center. This study was approved by the Institutional Review Board of the University of Illinois at Chicago. All subjects gave informed consent for the study. During hospitalization, the patients were kept drug-free for up to two weeks before they started treatment. Blood samples were drawn from the patients (n=31) in the morning under a fasting state. The clinical assessments were performed at the end of the drug-free period before initiation of treatment.

The NC subjects were non-hospitalized subjects who were recruited for the study through advertisements on hospital notice boards or by referral from normal controls or by referral from hospital employees. Control subjects had no history of psychiatric or major medical disorders and they abstained from any medication for at least two weeks before assessment or blood drawing.

2.2 Clinical assessment

Patients were diagnosed 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 BP depressed patients were not included in the study. The discharge diagnosis was considered definitive. Symptom ratings included scores on the Hamilton Depression Rating Scale (HDRS).

2.3 Blood processing

For each participant, 30ml of venous blood was collected in the morning into tubes containing 3.8% (w/v) sodium citrate in DEPC treated water and (1 vol: 9 vol blood) for plasma. The samples were centrifuged immediately at 210g for 15 min and 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 samples were then centrifuged at 400 g for 40 min. The upper layer above the interface layer was discarded and the interface layer was processed for lymphocyte isolation. The isolated

lymphocytes are stored at -80°C until assayed. Plasma was obtained by centrifuging the PRP secretion at 6000g for 10 minutes.

2.4 RNA isolation

Total RNA was extracted, from lymphocytes as previously described (Pandey et al., 2015), using TRIZOL reagent (Invitrogen) and DNase treatment were performed for each sample. The RNA concentration and purity was determined by measuring the optical density at 260-nm wavelengths using NanoDrop®ND-1000 (NanoDrop Technologies, Montchanin, DE, USA) and 260/280 nm ratio with expected values between 1.8 and 2.0. RNA quality was assessed using Agilent Bioanalyzer 2100 (Agilent). All samples used had 28S/18S ratios >1.2 , and RNA integrity number (RIN) above 6.6 with mean RIN values of 8.1 ± 0.7 .

cDNA Synthesis was synthesized as previously described (Pandey et al., 2015), briefly, 1 μg 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 . Thermal cycle conditions were 37°C for 60 min, 70°C for 15 minutes and stored at -20°C immediately. Real-time PCR was performed in duplicates using MX3005p sequence detection system (Agilent). Pre-designed Taqman gene expression assays (Applied Biosystems, Foster City, CA) were used for all target and internal control genes, description is given in Table 1.

The geNorm algorithm (Vandesompele et al., 2002) was used to determine stability and optimal numbers of internal controls, the two parameters used are: gene stability: M (average expression stability) and V (pair wise variation), where a low M value signifies a more stable gene and a V value of 0.15 is the proposed cut-off below which the inclusion of an additional gene is not necessary, of the 12 genes tested in our sample set, ACTB and GAPDH were identified to be the most stable internal controls. PCR efficiency for all genes, after 5-log dilution series of pooled cDNA, was similar. Each reaction was carried out using 10 μl of cDNA (diluted 1:10) in 1X TaqMan Universal PCR Master Mix (Applied Biosystems) as per 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. Q-RT-PCR data were analyzed using the $2^{-\Delta\text{Ct}}$ method, where $\Delta\text{Ct} = (\text{CT}_{\text{target}} - \text{CT}_{\text{internal control}})_{\text{Subject}} - (\text{CT}_{\text{target}} - \text{CT}_{\text{internal control}})_{\text{Control}}$, and $\text{CT}_{\text{internal control}}$ is the geometric mean of ACTB and GAPDH CTs. For further statistical analysis $2^{-\Delta\text{Ct}}$ values are used.

2.5 mRNA determination

Expression levels of mRNA were determined using a two-step real-time RT-PCR (qPCR) method. One μg 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, Foster City, CA) description is given in Table 1. The stability and optimal number of housekeeping genes was determined using geNORM version 3.4 (PrimerDesign Ltd, UK) according to the manufacturer's instructions (Vandesompele et al., 2002). 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 MasterMix (Applied Biosystems) 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. Relative expression levels, reported as fold change, were determined by the $2^{-(C_t)}$ method, where $CT = (CT_{\text{target}} - CT_{\text{normalizer}})_{\text{subject}} - (CT_{\text{target}} - CT_{\text{endogenous gene}})_{\text{control}}$ (Applied Biosystems User Bulletin No. 2). CT values are used for further statistical analysis.

2.6 Determination of plasma protein levels using ELISA

Levels of proinflammatory cytokines were determined in plasma aliquots by ELISA using commercially available Quantakine® kits (R & D Systems, Inc., Minneapolis, MN) for IL-1 β , IL-6 and TNF- α . Briefly, 100 µl of incubation buffer and 100 µL of serum/plasma or standard is added to each well and incubated for 3 hours at room temperature (RT) on the orbital shaker. After washing wells 6 times with Wash Buffer, 200 µL of Conjugate is added to each well, incubated for 2 hours at RT, washed using Wash Buffer as before, 50 µL of Substrate Solution is added to each well and incubated for 60 minutes at RT. Following this, 50 µL of Amplifier Solution is added to each well, incubate for 30 minutes at RT and 50 µL of Stop Solution is added to each well. The optical density (O.D.) of each well is determined within 30 minutes using a microplate reader set to 490 nm and wavelength correction set to 650 nm and the levels of cytokines are calculated. The standard curve was generated by plotting the mean absorbance for each standard and data points are linearized. The concentration of cytokines in each sample was determined by reading it against the standard curve.

2.7 Statistical analysis

We analyzed the data using SAS 9.2 statistical software package. First we used two sample t-test to compare NC subjects with MDD 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 behavioral rating scores and the cytokine mRNA and protein measures.

3. Results

3.1 Demographic and clinical characteristics

The demographic and clinical characteristics of the MDD patients and NC subjects are provided in Table 2. When we compared the mean age of the subjects, we did not find any significant difference in the mean age of MDD patients and NC subjects. Gender and race distribution were very similar between the two groups. The mean in-hospital, drug-free period was 6.12 ± 2.03 days for the MDD patients. The mean HDRS score for MDD patients was 31.4 which is similar to that reported in literature.

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

The mRNA levels of membrane bound receptors subtypes of IL-1, TNF- α , and IL-6 were determined in the lymphocytes of MDD patients and NC subjects. When we compared the mRNA expression levels of these receptors in MDD patients and NC subjects, we observed that the mRNA levels of IL1R1 and IL1RA were significantly increased in MDD patients as compared to normal control subjects (Figure 1, Table 3). However, the mRNA levels of IL1R2 were not significantly different in MDD patients compared to NC subjects. When we compared the mRNA levels of TNFR1 and TNFR2, we found that the TNFR1 and TNFR2 levels were increased in the MDD patients as compared to NC subjects (Figure 1, Table 3). We also determined the mRNA levels of IL6R and IL6STU which is also known as Gp130. However, we did not find any significant difference in the mRNA levels of IL6R or GP130 between MDD patients and NC subjects as shown in Figure 1 and Table 3.

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

In order to examine if the mRNA levels of proinflammatory cytokines are changed in MDD patients, we also determined the mRNA levels of IL-1 β , IL-6, and TNF- α in lymphocytes of MDD patients and NC subjects. When we compared the mean mRNA levels in MDD patients and NC subjects, as shown in Figure 2 and Table 3, we found that the mRNA levels of proinflammatory cytokines IL-1 β , IL-6, and TNF- α were significantly increased in the lymphocytes of MDD patients as compared to NC subjects.

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

In order to examine if there was also a change in protein levels of proinflammatory cytokines, we determined the protein levels of the proinflammatory cytokines IL-1 β , IL-6, and TNF- α in the plasma of MDD patients and NC subjects. We found that the mean protein levels of IL-1 β , IL-6, and TNF- α was significant higher in the MDD patients as compared to the NC subjects (Figure 3, Table 4), suggesting that similar to the mRNA levels, the protein levels of the proinflammatory cytokines were also increased in the MDD patients as compared to NC subjects. The results are shown in Figure 3 and Table 4.

3.5 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 to be significant for mRNA measure IL-1R2, IL-6ST (i.e., Gp130), and race was significant for mRNA measure IL-6R and protein measure IL-1 β . Overall the results for GLM approach matched with t-test results stated before. To examine the association between group and gender we performed a contingency chi-square test and found no significant association. There was no significant association between any of the cytokine measures and HDRS scores in MDD patients.

4. Discussion

We determined the mRNA levels of proinflammatory cytokines and their membrane-bound receptors in the lymphocytes of MDD patients and NC subjects. We observed that the mRNA levels of IL-1 β , IL-6, and TNF- α were significantly higher in the lymphocytes of MDD patients as compared to NC subjects. We also observed that the mRNA levels for IL1R1 and the IL1RA, TNFR1 and TNFR2, were significantly increased in the lymphocytes of depressed patients as compared to NC subjects. However, we did not find any significant difference in the mRNA levels of IL1R2, IL6R, and Gp130 in the lymphocytes of MDD patients. When we compared the protein levels of the proinflammatory cytokines between MDD patients and NC subjects, we found that similar to the mRNA levels, the protein levels of IL-1B, IL-6, and TNF- α were significantly increased in the plasma of MDD patients as compared to NC subjects.

Therefore, in summary, our studies indicate increased expression of proinflammatory cytokines in the lymphocytes, and increases in specific subtypes of receptors of proinflammatory cytokines in the lymphocytes of depressed patients, suggesting that not only the proinflammatory cytokines, but also their membrane-bound receptors are abnormally expressed in depression.

To our knowledge, this is the first study of membrane-bound receptors of proinflammatory cytokines in blood cells of depressed patients, although membrane-bound cytokine receptors have been studied in human postmortem brain (Shelton et al., 2011).

We recently reported the studies of protein and mRNA levels of proinflammatory cytokines and their receptors in the plasma and lymphocytes of BP patients. A comparison of our studies in BP patients (Pandey et al., 2015) and depressed patients show many similarities but also some differences. For example, we found that the protein and mRNA levels of IL-1 β , IL-6 and TNF- α were significantly increased in both BP (Pandey et al., 2015) and in MDD patients (current study). However, we also found that whereas the mRNA levels of IL-1R1, IL-1RA and TNFR1 were increased in lymphocytes of both BP (Pandey et al., 2015) and MDD patients, the mRNA levels of TNFR2 were significantly increased in depressed patients compared to controls, but were not significantly different in BP patients compared with NC subjects. Thus, our studies of MDD and BP patients indicate common

abnormalities of proinflammatory cytokines and most of their receptors with the exception of TNFR2.

The receptors for the cytokines exist in two forms, the cell surface or the signal transducing receptors and the soluble form of the cytokine receptors. The soluble form of cytokine receptors represent truncated forms of the membrane-bound receptors that lack transmembrane and intra-cytoplasmic domains (Aderka et al., 1992; Fernandez-Botran, 1991). These soluble receptors may arise from proteolytic cleavage of the membrane-bound receptors or by synthesis from separate alternatively spliced mRNAs coding for soluble versus membrane forms (Cortez-Cooper et al., 2013; Fernandes et al., 2011).

In addition to the membrane-bound receptors, and soluble receptors, soluble receptor antagonists have also been reported for the cytokines, especially for the IL-1 receptors (Fernandez-Botran, 1991). These receptor antagonists bind specifically to the cytokines receptor but are themselves devoid of any biological activity. They produce their effects by competing with the biologically active cytokine for binding to the same membrane-bound receptor (Hannum et al., 1990). To date, the only example of this kind of receptor antagonist is the human IL-1RA described by Hannum et al. (1990) and cloned by Eisenberg et al. (1990). Cytokines produce their biological and behavioral effects by acting on their membrane-bound receptors, i.e., the cell surface form of cytokine receptors (Hohmann et al., 1989). These receptors function as transducing elements producing molecular signals of cytokines within cells through several signal transduction pathways (Park and Bowers, 2010; Santello and Volterra, 2012). Specific subtypes of receptors have been cloned and identified for the proinflammatory cytokines. Thus, for IL-1 two types of receptors have been cloned, known as IL-1R1 and IL-1R2 (McMahan et al., 1991; Sims et al., 1989), of which only IL-1R1 initiates signal transduction while IL-1R2 presumably functions only as a ligand sink or as a decoy receptor (Sims et al., 1993). Thus, most of the IL-1 signal has been shown to be transmitted through IL-1R1.

TNF- α is a major mediator of apoptosis as well as immunity and inflammation (Aggarwal, 2003; Chen and Goeddel, 2002). Inappropriate production of TNF- α or sustained activation of TNF signaling has been associated in pathogenesis of many human diseases including depression (Clark et al., 2010; Simen et al., 2006). TNF- α and TNF- β produce their intracellular signals and biological effects by binding with high affinity to two distinct subtypes of receptors known as TNFR1 and TNFR2 (Park and Bowers, 2010; Santello and Volterra, 2012). Although these two receptors also exist in soluble forms, only membrane-bound TNF receptors function as transducing elements able to produce intracellular molecular signals (Park and Bowers, 2010; Santello and Volterra, 2012).

The biological effects mediated by TNF receptors are apoptosis, cell survival, differentiation or proliferation through the activation of pathways involving nuclear factor *kappa beta* (NF- κ B), Jun-N-terminal kinase, and mitogen-activated protein (MAP) kinase (Park and Bowers, 2010; Santello and Volterra, 2012). However, the signaling mechanisms mediated by TNFR1 and TNFR2 are complex, for details see Ihnatko and Kubes (2007), Chen and Goeddel (2002).

The binding of TNF to TNFR1 triggers a series of intracellular events that result in the activation of two major transcription factors, NF- κ B and C-Jun. The activation of caspases (downstream signaling) causes apoptosis and the activation of NF- κ B sends cell survival signals (Park and Bowers, 2010; Santello and Volterra, 2012).

In our studies we observed an increase in both TNFR1 and TNFR2 expression in the lymphocytes of depressed patients suggesting that the alteration of biological and behavioral effects in depressed patients may be related to the increased levels of both TNFR1 and TNFR2.

The cellular signaling of IL-6 is initiated by its binding to IL-6R forming an IL-6/IL-6R heterodimer which then associates with Gp130 leading to the activation of various signaling pathways but predominantly activates the Janus kinase/signal transduction and activation of transcription (JAK/STAT) pathway and mitogen activated protein (MAP) kinase pathways (Rose-John, 2003; Rose-John et al., 2006). Although we found significantly higher levels of IL-6 in the lymphocytes of depressed patients, the levels of IL-6R or Gp130 were not significantly altered in the lymphocytes of depressed patients compared with controls.

Protein expression of proinflammatory cytokines and their soluble receptors in depression have been studied by several investigators (Hiles et al., 2012; Howren et al., 2009; Liu et al., 2012), as reviewed by Dowlati et al. (2010). A meta-analysis of these studies suggest that, in general, the levels of IL-1 β , IL-6, and TNF- α are increased in the plasma of depressed patients (Dowlati et al., 2010). Although not always consistent, these studies also indicate an increase in the levels of soluble receptors for these cytokines, IL-1R1 and TNFR1 and TNFR2. Recently, Cattaneo et al. (2013) reported increased leukocyte mRNA levels in depressed patients compared with normal controls. Our results of gene and protein expression of IL-1 β , IL-6, and TNF α are thus consistent with the previous reports. We also found increased levels of IL-1R1, IL-1RA, TNFR1, and TNFR2. Since the soluble receptors are formed by proteolytic degradation of membrane-bound receptors, it is quite possible that the increase in soluble receptors may be related to increased levels of membrane-bound receptors or receptor antagonist, such as IL-1 RA.

Although circulating levels of proinflammatory cytokines and their soluble receptors have been studied in MDD patients (Schiepers et al., 2005), both in relation to their role in its pathophysiology or as biomarkers (Schmidt et al., 2011), gene expression studies of these cytokines may be a better means to study their role in pathophysiology and as biomarkers or predictors of clinical response (Le-Niculescu et al., 2013; Menke, 2013), for the following reasons. Circulating concentrations of cytokines in the plasma are low due to their higher bioactivity and short half-life. In contrast, the mRNA levels in white cells are more stable and their concentrations are much higher, as shown by our results and those of Padmos et al. (2008). The magnitude of the difference in mRNA levels between MDD patients and NC subjects observed by us is much greater compared with the magnitude of difference in protein levels between MDD and NC subjects. The mRNA levels of cytokine receptors may be even better biomarkers because they are more stable and are involved in mediating the functional effects of cytokines through cellular signaling mechanisms (Aderka et al., 1992) and thus producing their physiological and behavioral effects.

4.1 Limitation

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.

4.2 Conclusion

To our knowledge, this is the first study of gene expression of membrane-bound cytokine receptors in depressed patients, although gene expression of proinflammatory cytokines have been reported in BP and schizophrenic patients (Drexhage et al., 2010; Padmos et al., 2008), and in depressed patients (Cattaneo et al., 2013). Studies of membrane-bound cytokine receptors may be important since they are involved in mediating the behavioral and functional effects of cytokines. These receptors are also important as targets for developing therapeutic agents for the treatment of depression, as has been done before by the use of TNF- α in the treatment of psoriasis patients (Tyring et al., 2006). Also, we observed that specific subtypes of receptors are abnormally expressed in the lymphocytes of depressed patients, and it may be more fruitful to target these abnormally expressed subtypes of cytokines receptors. Since this is the first study of cytokine receptors in MDD patients it needs to be replicated by other investigators.

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Abbreviations

BP	bipolar
BMI	body mass index
CNS	central nervous system
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
ELISA	enzyme-linked immunosorbent assay
GLM	generalized linear model
Gp130	glycoprotein130
HDRS	Hamilton depression rating scale
IL	interleukin
IL-1R	interleukin-1 receptor
IL-1RA	interleukin-1 receptor antagonist

IL-6ST	Interleukin-6 signal transducer
MAP kinase	mitogen activated protein (MAP) kinase
MDD	Major depressive disorder
NC	normal controls
PRP	platelet-rich plasma
qPCR	real-time RT-PCR
RIN	RNA integrity number
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor

References

- Aderka D, Engelmann H, Maor Y, Brakebusch C, Wallach D. Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. *J Exp Med*. 1992; 175:323–329. [PubMed: 1310100]
- Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol*. 2003; 3:745–756. [PubMed: 12949498]
- Capuron L, Ravaud A, Gualde N, Bosmans E, Dantzer R, Maes M, Neveu PJ. Association between immune activation and early depressive symptoms in cancer patients treated with interleukin-2-based therapy. *Psychoneuroendocrinology*. 2001; 26:797–808. [PubMed: 11585680]
- Capuron L, Ravaud A, Miller AH, Dantzer R. Baseline mood and psychosocial characteristics of patients developing depressive symptoms during interleukin-2 and/or interferon-alpha cancer therapy. *Brain Behav Immun*. 2004; 18:205–213. [PubMed: 15050647]
- Cattaneo A, Gennarelli M, Uher R, Breen G, Farmer A, Aitchison KJ, Craig IW, Anacker C, Zunsztain PA, McGuffin P, Pariante CM. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline ‘predictors’ and longitudinal ‘targets’. *Neuropsychopharmacology*. 2013; 38:377–385. [PubMed: 22990943]
- Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. *Science*. 2002; 296:1634–1635. [PubMed: 12040173]
- Clark IA, Alleva LM, Vissel B. The roles of TNF in brain dysfunction and disease. *Pharmacol Ther*. 2010; 128:519–548. [PubMed: 20813131]
- Cortez-Cooper M, Meaders E, Stallings J, Haddow S, Kraj B, Sloan G, McCully KK, Cannon JG. Soluble TNF and IL-6 receptors: indicators of vascular health in women without cardiovascular disease. *Vasc Med*. 2013; 18:282–289. [PubMed: 24165468]
- Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. *Ann N Y Acad Sci*. 2001a; 933:222–234. [PubMed: 12000023]
- Dantzer R. Cytokine-induced sickness behavior: where do we stand? *Brain Behav Immun*. 2001b; 15:7–24. [PubMed: 11259077]
- Dantzer, R.; Aubert, A.; Bluthé, R-M.; Gheusi, G.; Cremona, S.; Laye, S.; Konsman, JP.; Parnet, P.; Kelley, KW. Mechanisms of the behavioral effects of cytokines. In: Dantzer, R.; Wollman, EE.; Yirmiya, R., editors. *Cytokines, stress and depression*. New York: Kluwer Academic/Plenum Publishers; 1999. p. 83-105.
- Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. *Brain Behav Immun*. 2007; 21:153–160. [PubMed: 17088043]
- Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctot KL. A meta-analysis of cytokines in major depression. *Biol Psychiatry*. 2010; 67:446–457. [PubMed: 20015486]

- Drexhage RC, Knijff EM, Padmos RC, Heul-Nieuwenhuijzen L, Beumer W, Versnel MA, Drexhage HA. The mononuclear phagocyte system and its cytokine inflammatory networks in schizophrenia and bipolar disorder. *Expert Rev Neurother*. 2010; 10:59–76. [PubMed: 20021321]
- Eisenberg SP, Evans RJ, Arend WP, Verderber E, Brewer MT, Hannum CH, Thompson RC. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. *Nature*. 1990; 343:341–346. [PubMed: 2137201]
- Fernandes BS, Gama CS, Cereser KM, Yatham LN, Fries GR, Colpo G, de Lucena D, Kunz M, Gomes FA, Kapczinski F. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. *J Psychiatr Res*. 2011; 45:995–1004. [PubMed: 21550050]
- Fernandez-Botran R. Soluble cytokine receptors: their role in immunoregulation. *FASEB J*. 1991; 5:2567–2574. [PubMed: 1868981]
- Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, Armes LG, Sommer A, Eisenberg SP, Thompson RC. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature*. 1990; 343:336–340. [PubMed: 2137200]
- Hiles SA, Baker AL, de Malmanche T, Attia J. A meta-analysis of differences in IL-6 and IL-10 between people with and without depression: exploring the causes of heterogeneity. *Brain Behav Immun*. 2012; 26:1180–1188. [PubMed: 22687336]
- Hohmann HP, Remy R, Brockhaus M, van Loon AP. Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha). *J Biol Chem*. 1989; 264:14927–14934. [PubMed: 2549042]
- Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med*. 2009; 71:171–186. [PubMed: 19188531]
- Ihnatko R, Kubes M. TNF signaling: early events and phosphorylation. *Gen Physiol Biophys*. 2007; 26:159–167. [PubMed: 18063842]
- Le-Niculescu H, Levey DF, Ayalew M, Palmer L, Gavrin LM, Jain N, Winiger E, Bhosrekar S, Shankar G, Radel M, Bellanger E, Duckworth H, Olesek K, Vergo J, Schweitzer R, Yard M, Ballew A, Shekhar A, Sandusky GE, Schork NJ, Kurian SM, Salomon DR, Niculescu AB 3rd. Discovery and validation of blood biomarkers for suicidality. *Mol Psychiatry*. 2013; 18:1249–1264. [PubMed: 23958961]
- Leonard BE, Myint A. The psychoneuroimmunology of depression. *Hum Psychopharmacol*. 2009; 24:165–175. [PubMed: 19212943]
- Liu Y, Ho RC, Mak A. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-alpha) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *J Affect Disord*. 2012; 139:230–239. [PubMed: 21872339]
- McMahan CJ, Slack JL, Mosley B, Cosman D, Lupton SD, Brunton LL, Grubin CE, Wignall JM, Jenkins NA, Brannan CI, et al. A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. *EMBO J*. 1991; 10:2821–2832. [PubMed: 1833184]
- Menke A. Gene expression: biomarker of antidepressant therapy? *Int Rev Psychiatry*. 2013; 25:579–591. [PubMed: 24151803]
- Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry*. 2009; 65:732–741. [PubMed: 19150053]
- Padmos RC, Hillegers MH, Knijff EM, Vonk R, Bouvy A, Staal FJ, de Ridder D, Kupka RW, Nolen WA, Drexhage HA. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. *Arch Gen Psychiatry*. 2008; 65:395–407. [PubMed: 18391128]
- Pandey GN, Ren X, Rizavi HS, Zhang H. Abnormal gene expression of proinflammatory cytokines and their receptors in the lymphocytes of patients with bipolar disorder. *Bipolar Disord*. 2015; 17:636–644. [PubMed: 26257203]
- Park KM, Bowers WJ. Tumor necrosis factor-alpha mediated signaling in neuronal homeostasis and dysfunction. *Cell Signal*. 2010; 22:977–983. [PubMed: 20096353]
- Rose-John S. Interleukin-6 biology is coordinated by membrane bound and soluble receptors. *Acta Biochim Pol*. 2003; 50:603–611. [PubMed: 14515143]

- Rose-John S, Scheller J, Elson G, Jones SA. Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J Leukoc Biol.* 2006; 80:227–236. [PubMed: 16707558]
- Santello M, Volterra A. TNFalpha in synaptic function: switching gears. *Trends Neurosci.* 2012; 35:638–647. [PubMed: 22749718]
- Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. *Progress in Neuro-psychopharmacology & Biological Psychiatry.* 2005; 29:201–217. [PubMed: 15694227]
- Schmidt HD, Shelton RC, Duman RS. Functional biomarkers of depression: diagnosis, treatment, and pathophysiology. *Neuropsychopharmacology.* 2011; 36:2375–2394. [PubMed: 21814182]
- Shelton RC, Claiborne J, Sidoryk-Wegrzynowicz M, Reddy R, Aschner M, Lewis DA, Mirnics K. Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Mol Psychiatry.* 2011; 16:751–762. [PubMed: 20479761]
- Simen BB, Duman CH, Simen AA, Duman RS. TNFalpha signaling in depression and anxiety: behavioral consequences of individual receptor targeting. *Biological Psychiatry.* 2006; 59:775–785. [PubMed: 16458261]
- Sims JE, Acres RB, Grubin CE, McMahan CJ, Wignall JM, March CJ, Dower SK. Cloning the interleukin 1 receptor from human T cells. *Proc Natl Acad Sci U S A.* 1989; 86:8946–8950. [PubMed: 2530587]
- Sims JE, Gayle MA, Slack JL, Alderson MR, Bird TA, Giri JG, Colotta F, Re F, Mantovani A, Shanebeck K, et al. Interleukin 1 signaling occurs exclusively via the type I receptor. *Proc Natl Acad Sci U S A.* 1993; 90:6155–6159. [PubMed: 8327496]
- Tyring S, Gottlieb A, Papp K, Gordon K, Leonardi C, Wang A, Lalla D, Woolley M, Jahreis A, Zitnik R, Cella D, Krishnan R. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet.* 2006; 367:29–35. [PubMed: 16399150]
- Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 2002; 3 RESEARCH0034.

Highlights

- We examined the role of membrane-bound receptors for proinflammatory cytokines in major depressive disorders (MDD), by determining their gene expression.
- The subtypes of receptors studied were IL-1R1, IL-1R2, IL-1RA, TNFR1, TNFR2, IL-6 and Gp130.
- mRNA for specific receptor subtypes were increased in the lymphocytes of MDD patients.
- Both protein and mRNA levels of IL-1 β , IL-6 and TNFR2 were increased in MDD patients.
- Thus, in addition to the proinflammatory cytokines, membrane-bound cytokine receptors may have a role in pathophysiology of MDD.

Figure 1.

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 MDD patients and NC subjects. The data are shown as fold change [$2^{-(\Delta\Delta C_t)}$] in mRNA levels. Values are fold change $\pm$ S.E.M.

* $p < 0.05$

Figure 2.

Mean mRNA expression levels of proinflammatory cytokines, interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α in the lymphocytes of patients with major depressive disorder (MDD) and normal control (NC) subjects. The data are shown as fold change $[2^{-(\Delta\Delta C_t)}]$ in mRNA levels. Values are fold change $\pm$ S.E.M.

* $p < 0.05$

Figure 3. Mean protein expression levels of IL-1 β , IL-6, and TNF- α in the plasma of MDD patients and NC subjects. Values are mean $\pm$ SD.
* $p < 0.05$

Table 1

TaqMan primers/probes used for qPCR analysis

	TaqMan accession	Probe location (exon boundry)	Assay function
ACTB	Hs99999903_m1	1–1	HK
GAPDH	Hs99999905_m1	3–3	HK
IL-1 β	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

ACTB, beta-actin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HK, housekeeping gene

Table 2

Demographic Characteristics of Adult Depressed Patients and Normal Control Subjects

Group	Age (Years)	Gender (M/F)	Race	HDRS
Normal Controls (n = 30)	34.6 ± 13.5	17 M / 13 F	5 Asian 7 Black 2 Hispanic 16 White	--
Depressed Patients (n = 31)	34.3 ± 10.1	12 M / 19 F	2 Asian 11 Black 2 Hispanic 16 White	31.4 ± 7.5

Values are the mean ± SD.

Abbreviations: HDRS, Hamilton Depression Rating Scale; F, female; M, male

Table 3

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

Variable	Normal Controls (n = 30)		Depressed Patients (n = 31)		t	p
	Mean	CT ± SD	Mean	CT ± SD		
TNF- α	9.06 ± 0.51		7.96 ± 0.34		- 9.86	<0.0001
IL-1 β	7.79 ± 0.48		6.67 ± 0.38		- 10.06	<0.0001
IL-6	13.65 ± 0.50		12.46 ± 0.53		- 8.26	<0.0001
TNFR1	4.89 ± 0.48		4.35 ± 0.61		- 3.82	0.0003
TNFR2	4.77 ± 0.32		4.41 ± 0.62		- 2.8	0.0068
IL-1R1	7.48 ± 0.54		7.18 ± 0.54		- 2.15	0.036
IL-1R2	5.76 ± 0.47		5.83 ± 0.51		- 0.66	0.51
IL-1RA	3.05 ± 0.37		2.44 ± 0.56		- 4.91	<0.0001
IL-6ST (Gp130)	7.32 ± 0.53		7.13 ± 0.63		-1.2	0.24
IL-6R	6.40 ± 0.53		6.20 ± 0.63		-1.23	0.22

Table 4

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

Variable	Normal Controls (n = 30) Mean (pg/ml) $\pm$ SD	Depressed Patients (n = 31) Mean (pg/ml) $\pm$ SD	t	p
TNF- α	1.46 $\pm$ 0.39	2.13 $\pm$ 1.49	2.60	0.01
IL-1 β	1.26 $\pm$ 0.81	2.58 $\pm$ 1.37	6.61	<.001
IL-6	0.99 $\pm$ 0.39	1.22 $\pm$ 0.46	-1.92	0.034