Higher Baseline Proinflammatory Cytokines Mark Poor Antidepressant Response in Bipolar Disorder

Francesco Benedetti, MD[a,*]; Sara Poletti, PhD[a]; Thomas A. Hoogenboezem, ing[b]; Clara Locatelli, MD[a]; Harm de Wit, ing[b]; Annemarie J. M. Wijkhuijs, ing[b]; Cristina Colombo, MD[c]; and Hemmo A. Drexhage, MD, PhD[b]

ABSTRACT

Background: The clinical relevance of raised levels of circulating cytokines in bipolar disorder is still unclear. Cytokines influence neurotransmitters, neuroplasticity, and white matter integrity. An inconsistent literature suggests that higher cytokine levels could hamper antidepressant response. Total sleep deprivation (TSD) and light therapy (LT) prompt a rapid antidepressant response and can provide a model treatment to study predictors of response.

Methods: We studied at baseline 15 immune-regulating compounds in 37 consecutively admitted inpatients with a major depressive episode in the course of bipolar disorder (*DSM-5* criteria) and in 24 controls. Thirty-one patients (84%) had a lifetime history of drug resistance. Patients were administered 3 TSD + LT cycles in 1 week (study period: 2010–2012). Data were analyzed with age- and false-discovery-rate–corrected analysis of variance and were tested as predictors in a regressive model.

Results: Twenty-three patients (62%) responded to treatment (Inventory of Depressive Symptomatology IDS-C score < 12). Five highly intercorrelated compounds (IL-8, MCP-1, IFN-γ, IL-6, TNF-α) showed higher levels in nonresponder patients as compared to responders, corrected for multiple comparisons (respectively $F = 6.138$, $P_{FDR} = .0134$; $F = 6.197$, $P_{FDR} = .0134$; $F = 4.785$, $P_{FDR} = .0255$; $F = 3.782$, $P_{FDR} = .0441$; $F = 3.764$, $P_{FDR} = .0441$). A principal component analysis identified a single component that explained 84% of variance of these cytokines ($Q^2 = 0.15$), and a high factor score significantly predicted worse response (b = –0.692; $W = 4.34$, $P = .037$). A higher body mass index correlated with higher cytokines ($r = 0.430$, $P = .010$), indirectly hampering response (b = –0.0192, $P = .013$).

Conclusions: Proinflammatory compounds reflecting an M1-like proinflammatory state of monocytes/macrophages are associated with a poor response to antidepressant TSD + LT treatment in bipolar depression.

J Clin Psychiatry 2017;78(8):e986–e993
https://doi.org/10.4088/JCP.16m11310

[a]Division of Neuroscience, Scientific Institute Ospedale San Raffaele, Milano, Italy
[b]Department of Immunology, Erasmus University Medical Centre, Rotterdam, Netherlands
[c]School of Medicine, University Vita-Salute San Raffaele, Milano, Italy
**Corresponding author:* Francesco Benedetti, MD, Istituto Scientifico Universitario Ospedale San Raffaele, Dipartimento di Neuroscienze Cliniche, San Raffaele Turro, Via Stamira d'Ancona 20, 20127 Milano, Italy (benedetti.francesco@hsr.it).

Evidence supports an increased production of proinflammatory cytokines, in the absence of active somatic immune diseases, in patients affected by mood disorders. The interpretation of this finding is still under debate, and several different mechanisms have been proposed, including an inborn dysregulation of the immune system leading to auto-inflammatory reactivity, stress, and exposure to infectious agents.[1–5]

Not all patients show the same immune dysregulation pattern, and the clinical relevance and consequences of having altered immune reactivity and inflammation are still unclear. A sparse literature in patients affected by major depressive disorder (MDD) suggests that a proinflammatory profile can influence the outcome of mood episodes. A pivotal study[6] suggested that unstimulated pretreatment production of interleukin 6 (IL-6) may be a predictor of response to antidepressant drug treatment with amitriptyline: in comparison with control subjects, basal IL-6 values were found to be lower in responders and higher in nonresponders. Similarly, basal plasma concentrations of acute-phase proteins (C-reactive protein, α_1-acid glycoprotein, and α_1-antichimotripsin) suggested a higher inflammatory activation in nonresponders to lithium potentiation of antidepressants, with lower inflammatory patterns in responders.[7] Increased serum IL-6 and interleukin 1 (IL-1) receptor antagonist concentrations marked resistance to antidepressant treatment.[8] Plasma IL-6, but not plasma tumor necrosis factors α (TNF-α), was higher in selective serotonin reuptake inhibitor (SSRI)– and serotonin and norepinephrine reuptake inhibitor (SNRI)–refractory depressed patients than in responders to treatment.[9] Higher levels of TNF-α, but not of other proinflammatory cytokines, associated with poor response to escitalopram in 1 study,[10] but with better response to combined exercise and antidepressants in another.[11] Higher serum levels of interleukin 2 (IL-2) receptor, but not of other cytokines, were reported in nonresponders to monoaminergic antidepressants.[12] Inconsistent and negative results have also been reported across different studies[13;] thus, a clear and consistent picture of the prediction capability of raised inflammation regulating factors in the serum of MDD patients is still lacking. Also, the possible clinical relevance of these markers in respect to treatment with conventional antidepressant drugs seems mainly limited to response prediction, because a meta-analysis[14] of 22 studies comprising 603 subjects did not find a clear relationship between changes of immunologic indices after treatment and antidepressant response.

Our group performed the only predictive studies available of patients affected by bipolar disorder and showed (1) an inverse relationship between baseline serum IL-6 and subsequent antidepressant response to sleep deprivation and sleep phase-advance,[15] and (2) a treatment-induced increase of stem cell factor, which is both a hematopoietic growth factor and a neurotrophic factor, involved in neuron-microglia interactions and in fostering an antiinflammatory milieu,

Clinical Points

- A consistent literature reported higher levels of immune-regulating cytokines in patients affected by mood disorders, but the clinical relevance of these findings is still unclear, with very few clinical studies available for bipolar disorder.
- Higher levels of 5 cytokines associated with a detrimental M1-like proinflammatory state (IL-8, MCP-1, IFN-γ, IL-6, and TNF-α) can predict poor response to antidepressant chronotherapy of bipolar depression. Body mass index correlated with higher cytokines, also indirectly hampering response.
- Evidence is sparse that antiinflammatory drugs can potentiate antidepressant response. This study suggests that stratification of patients with bipolar depression on the basis of immune signatures could help to select a specific treatment-resistant subgroup that could benefit from the therapeutic targeting of the activated inflammatory response system in everyday clinical practice.

positively correlating with response to treatment.[16] Altered cytokine levels are expected in bipolar disorder during both symptomatic and euthymic intervals.[17,18] Raised peripheral cytokine levels are currently considered as part of a more general perturbation of the immune system, including higher macrophage/monocyte inflammatory activation patterns (which leads to high levels of proinflammatory and antiinflammatory monocyte/macrophage–derived cytokines) and paralleled by reduced T cell activity.[3]

The depressive phase of bipolar illness is also associated with marked disturbances of the sleep-wake cycle and can be treated with somatic therapies that target sleep and circadian rhythms (chronotherapeutics) to cause marked and rapid clinical improvements, such as total sleep deprivation (TSD), alone or combined with light therapy (LT).[19,20] The need for the rapid resolution of depression,[21] and for the prevention of both treatment-emergent mania[22,23] and relapse after treatment discontinuation,[24] can lead to highly complex medication regimens in bipolar disorder.[25] When combined with lithium, chronotherapeutics skips the long latencies of traditional antidepressant treatments while providing a comparable efficacy with lower risk of inducing manic switches,[20,26–28] causing a stable remission of bipolar depression in more than half of the treated patients[29–31] and the rapid resolution of the worse depressive symptoms, such as suicide.[32] The rapidity of effects of chronotherapeutics enables the study of the biological correlates of response at close time points, thus providing a good model to disentangle the contribution of single biological factors affecting antidepressant response in the absence of the confounding factors linked with antidepressant drugs.[33–38]

The biological bases of the phenomenon of nonresponse to antidepressant treatments are largely unknown, hence the interest in the immune state as a determinant of therapy outcome. Insight in such processes could speed up the investigation of new treatments promoting the identification of new drug targets.[39] The paucity of literature studies about the possible predictive value of inflammation and immune regulation markers in bipolar disorder warrants interest for further investigations in the field. With an exploratory approach, we studied the relationship between a panel of inflammation-related and immune-activation–related cytokines with the antidepressant response to TSD + LT in bipolar depression to test the hypothesis that higher inflammation and immune activation markers could associate with worse antidepressant response.

METHODS

Participants

We studied 37 consecutively admitted inpatients (study period: 2010–2012) affected by a major depressive episode, without psychotic features, in the course of bipolar disorder type I (*DSM-5* criteria). Twenty-four healthy participants, recruited by advertisement in the university, served as controls. Inclusion criteria were to be willing to participate; absence of other diagnoses on Axis I, pregnancy, history of epilepsy, major medical and neurologic disorders; no treatment with long-acting neuroleptic drugs in the previous 3 months before admission; and absence of a history of drug or alcohol dependency or abuse within the previous 6 months. The study was performed within the frame of the MOODINFLAME project (http://moodinflame.eu/), a large-scale, European, medical scientific project aiming to advance early diagnosis, treatment, and prevention of mood disorders targeting the activated inflammatory response system. Additional MOODINFLAME exclusion criteria were inflammation-related symptoms, including fever and infectious or inflammatory disease; uncontrolled systemic disease; uncontrolled metabolic disease or other significant uncontrolled somatic disorder known to affect mood; somatic medications known to affect mood or the immune system, such as corticosteroids, nonsteroid antiinflammatory drugs, and statins. Physical examinations, laboratory tests, and electrocardiograms were performed at admission. After presenting a complete description of the study to the subjects, a written informed consent was obtained. All the research activities were approved by the local ethics committee.

Treatment

Thirty-one patients (84%) had a positive lifetime history of drug resistance according to Thase and Rush criteria[40]: 10 (27%) were resistant to 1 class of drugs, 14 (38%) to 2 classes, and 7 (19%) had higher stages of resistance. All patients were administered 3 consecutive TSD cycles (days 0–7); each cycle was composed of a period of 36 hours awake. On days 1, 3, and 5 patients were totally sleep deprived from 7 AM until 7 PM of the following day. They were then allowed to sleep during the nights of days 1, 3, and 5. Patients were administered LT (exposure for 30 minutes to a 10,000-lux bright white light, color temperature 4,600 K) at 3 AM during the TSD night and in the morning after recovery sleep, half an hour after awakening, between 8 AM and 9 AM. Light therapy in the morning was administered for 2 weeks. Patients were

Table 1. Clinical and Demographic Characteristics of the Patients Divided According to Final Response to Treatment[a,b]

Characteristic	Responders[c] (n = 23)	Nonresponders (n = 14)	*F* or χ^2	*P*
Age, y	44.09 ± 15.12	48.86 ± 13.44	15.37	.34
Sex (male/female)	6/17	4/10	0.03	.87
Age at onset of illness, y	31.04 ± 12.82	33.00 ± 12.41	0.21	.65
Duration of illness, y	12.74 ± 7.46	15.86 ± 11.22	1.04	.32
Duration of current episode, wk	25.52 ± 24.71	36.57 ± 42.19	0.96	.34
Number of previous episodes				
Depressive episodes	6.00 ± 6.76	8.62 ± 7.32	1.17	.29
Manic episodes	3.43 ± 6.21	4.23 ± 6.52	0.13	.72
Body mass index, based on kg/m^2	24.20 ± 4.20	27.05 ± 5.43	3.14	.085
Childhood Trauma Questionnaire, score	36.35 ± 8.24	40.08 ± 12.41	0.95	.34
IDS-C score				
At baseline	33.30 ± 10.00	33.57 ± 12.12	0.005	.94
After treatment	5.43 ± 3.82	25.43 ± 11.11	63.25	< .00001

[a]All values are mean ± SD unless otherwise noted.
[b]Healthy controls (n = 24) were significantly younger than patients (27.50 ± 9.96 vs 45.89 ± 14.51 years; $t = 5.42$, $P < .001$), but had the same sex distribution (male/female = 9/15; $\chi^2 = 0.74$, $P = .38$)
[c]Responders were those who had an IDS-C score < 12.
Abbreviation: IDS-C = clinician-rated Inventory of Depressive Symptomatology.

either taking lithium at admission, and continued it (n = 8), or started lithium together with the chronotherapeutic procedure to enhance its effect and prevent relapse.[32,41] No other antidepressant was administered.

Blood sampling for cytokine assessment was performed in the morning of the day before the start of treatment (day 0). Severity of depression was rated (day 0 and 7) on the clinician-rated Inventory of Depressive Symptomatology (IDS-C).[42] Response to treatment was categorically defined according to the strict remission criterion of a final IDS-C score < 12.[43] Twenty-nine patients rated early traumatic experiences on the Childhood Trauma Questionnaire (CTQ).[44]

Laboratory Determinations

We selected a panel of 15 compounds that have been linked to mood disorders[1–3] and that could reliably be measured with our current assays (results were well within the range of the standard curves and above the detection limits of the assays used). They included (1) proinflammatory or antiinflammatory activation factors linked to innate immune cells: TNF-α, IL-6, interleukin 8 (IL-8), interleukin 10 (IL-10), pentraxin-related protein 3 (PTX3), and interleukin 1 receptor antagonist (IL-1Ra); (2) factors linked to proinflammatory and antiinflammatory T cell activation: interferon γ (IFN-γ) and interleukin 5 (IL-5); (3) compounds reflecting the chemotactic activity of immune cells: monocyte chemotactic protein 1 (MCP1) and C-X-C motif chemokine 10 (CXCL10); (4) compounds reflecting the differentiation capacity of immune cells: interleukin 7 (IL-7), interleukin 2 receptor α (IL-2RA), and granulocyte-colony stimulating factor (G-CSF); and (5) compounds reflecting the adhesion activity of immune cells: vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1).

Serum concentrations of TNF-α, IL-6, IL-8, IL-10, IL-1Ra, IFN-γ, IL-5, IL-7, MCP1, and CXCL10 were measured using a bead-based Luminex system. These multiplexed sandwich immunoassays were developed from commercially available capture and detection antibodies and standard proteins, validated and approved at EDI GmbH, Karlsruhe (Pfinztal), Germany, according to methods described previously.[45] Assays were measured on either the Luminex FlexMap-3D or Luminex 200 system. PTX3, G-CSF, VCAM-1, ICAM-1, and IL-2RA levels were analyzed using a commercially available ELISA kit (BDNF Quantikine ELISA, R&D Systems, Abingdon, United Kingdom). Samples were analyzed in duplicates according to manufacturer's instructions. The intraassay coefficient of variation was 3.8%–6.2%, and the interassay coefficient of variation was 7.6%–11.3%.

Data Analysis

Levels of each circulating cytokine were compared between the 3 groups of (1) patients responding to treatment, (2) patients not responding to treatment, and (3) healthy controls by means of analyses of variance with Newman-Keuls post hoc tests. Analyses were performed in the context of the general linear model (GLM).[46,47] The significance of the effect of the single independent factor on each dependent variable was estimated (least squares method) by parametric estimates of predictor variables and following standard computational procedures.[48] Levels of significance of the observed differences were corrected for multiple comparisons with the method of the adaptive linear step-up procedures that control the false discovery rate (FDR).[49] Age was always included as nuisance covariate.

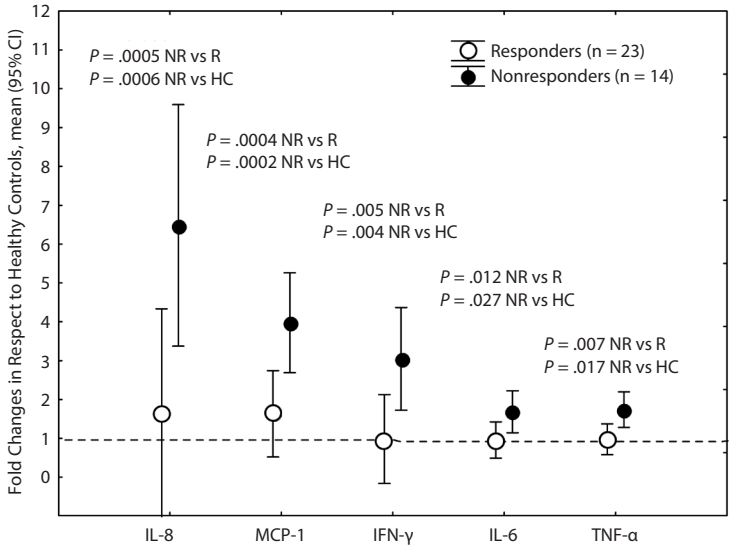
The possible predictive effect of the cytokines on antidepressant response was then tested by using the levels of circulating cytokines that significantly differed according to response as independent factors. Given that a high intercorrelation of predictors was expected, multicollinearity was diagnosed by calculating the variance inflation factor (VIF). In the presence of a mean VIF substantially > 1, which indicates that predictors have linear relationships among themselves that might bias regression,[50] multicollinearity was corrected by principal component analysis (PCA).[51,52] PCA was performed to identify orthogonal directions of maximum variance in the original data and to project the

Table 2. Levels of Circulating Cytokines and Direction and Levels of Significance of the Observed Differences[a]

Cytokine	Responders (R) (n = 23)	Nonresponders (NR) (n = 14)	Healthy Controls (HC) (n = 23)	*F*	FDR-Adjusted *P* Values[b]	Direction of Significant Effects[c]
IL-10 (pg/mL)	49.13 ± 60.97	75.01 ± 64.09	2.47 ± 2.04	9.177	.0038	R and NR > HC
IL-8 (pg/mL)	106.86 ± 126.68	471.98 ± 597.83	81.15 ± 69.56	6.138	.0134	NR > R and HC
MCP-1 (pg/mL)	60.02 ± 40.47	147.89 ± 113.70	37.19 ± 25.96	6.197	.0134	NR > R and HC
ICAM-1 (ng/mL)	192.44 ± 154.67	185.62 ± 70.17	59.13 ± 23.50	5.806	.0134	R and NR > HC
IFN-γ (pg/mL)	37.32 ± 40.25	119.82 ± 133.83	43.81 ± 52.21	4.785	.0255	NR > R and HC
IL-6 (pg/mL)	4.14 ± 3.00	7.62 ± 5.56	4.96 ± 2.53	3.782	.0441	NR > R and HC
TNF-α (pg/mL)	10.52 ± 5.15	17.14 ± 10.48	10.42 ± 6.51	3.764	.0441	NR > R and HC
PTX3 (pg/mL)	3,412.17 ± 2,656.23	4,944.05 ± 3,455.20	2,687.35 ± 1,603.83	2.543	.1151	…
CXCL10 (pg/mL)	44.58 ± 20.90	55.66 ± 41.98	28.12 ± 9.23	2.165	.1450	…
IL-1Ra (pg/mL)	466.70 ± 564.78	157.25 ± 121.65	346.31 ± 381.18	1.908	.1679	…
IL-2RA (pg/mL)	6,82.57 ± 305.29	840.19 ± 238.83	631.53 ± 237.54	1.111	.3212	…
IL-5 (pg/mL)	0.33 ± 0.29	0.20 ± 0.12	0.40 ± 0.47	0.787	.4035	…
IL-7 (pg/mL)	2.51 ± 2.28	3.71 ± 2.15	3.17 ± 2.53	0.621	.4388	…
G-CSF (pg/mL)	18.08 ± 19.06	20.05 ± 19.51	16.63 ± 6.72	0.108	.6376	…
VCAM-1 (ng/mL)	1,128.66 ± 535.40	1,076.41 ± 449.40	1,056.55 ± 312.86	0.093	.6376	…

[a]All values are mean ± SD unless otherwise noted. Ellipses (…) represent nonsignificant effects.
[b]*P* values are false discovery rate (FDR)–corrected for multiple comparisons. Fold changes are shown in Figure 1.
[c]Post hoc Newman-Keuls test.
Abbreviations: CXCL10 = C-X-C motif chemokine 10, G-CSF = granulocyte-colony stimulating factor, ICAM-1 = intercellular adhesion molecule 1, IFN-γ = interferon γ, IL-1Ra = interleukin-1 receptor antagonist, IL-2RA = interleukin 2 receptor α, IL-5 = interleukin 5, IL-6 = interleukin 6, IL-7 = interleukin 7, IL-8 = interleukin 8, IL-10 = interleukin 10, MCP1 = monocyte chemotactic protein 1, PTX3 = pentraxin-related protein 3, TNF-α = tumor necrosis factor α, VCAM-1 = vascular cell adhesion molecule 1.

Figure 1. Differences in the Peripheral Levels of the Cytokines, Which Significantly Differed in Nonresponders Compared With Antidepressant Treatment[a]



[a]For visualization purposes, data are shown as fold changes, with the line at y = 1 marking the level of the corresponding cytokine in healthy controls (see Table 2 for raw data in the studied groups). Levels of significance of the observed differences refer to post hoc Newman-Keuls critical ranges test.
Abbreviations: HC = healthy controls, NR = nonresponders, R = responders, IFN-γ = interferon γ, IL-6 = interleukin 6, IL-8 = interleukin 8, MCP1 = monocyte chemotactic protein 1, TNF-α = tumor necrosis factor α.

data into a lower-dimensionality space formed of a subset of the highest-variance component(s), detected according to the least squares criterion. In the presence of significant effects and eigenvalues for the component(s) > 1, the score of the component was then extracted and used as an independent factor to test the combined effect of the original collinear predictors on response.[53] Given that the distribution of response is binomial, this analysis was performed in the context of the generalized linear model (GLZM) with a logit link function.[54]

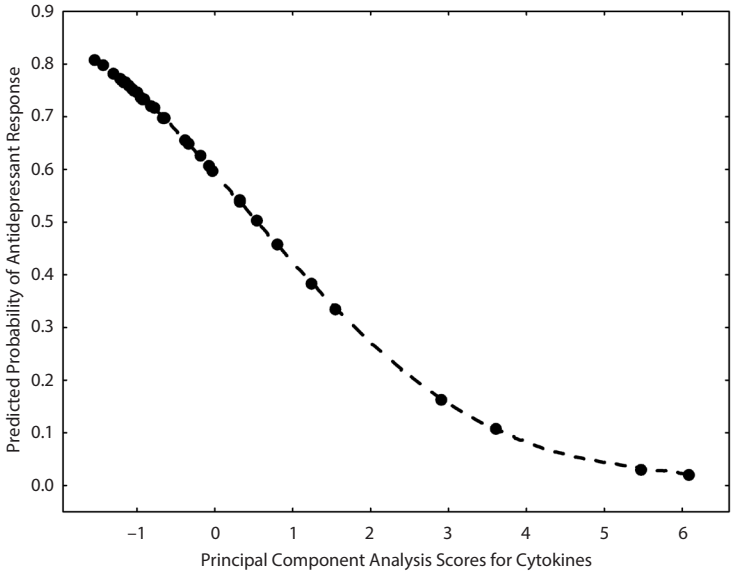
Furthermore, given that obesity associates with a state of chronic low-grade inflammation,[55] we investigated the effect of body mass index (BMI) both on cytokine levels and on observed and predicted response to treatment. To test the hypothesis that cytokines may mediate the possible effects of BMI on response to treatment, we performed a multiple regression mediation analysis with the PROCESS macro for SPSS (IBM Corporation) and SAS (SAS Institute), version 2.16,[56] using κ^2 estimate of effect size,[57] and a partial posterior method to test the significance of the indirect effect.[58]

RESULTS

Confirming expectations based on available literature, 23 patients (62.2%) responded to treatment and did not significantly differ from nonresponders on any clinical and demographic characteristic (Table 1).[30,32]

Inspection of data (Table 2) shows that nonresponders had higher mean values of most of the studied analytes, with high within-group variability for all measures. Age-corrected analyses of variance showed that 7 of 15 cytokines significantly differed among groups, surviving the statistical threshold of $P < .05$ FDR-corrected for multiple comparisons. Two cytokines (IL-10 and ICAM-1) showed an effect of diagnosis, with higher values in patients, both responders and nonresponders, compared to controls. Five cytokines (IL-8, MCP-1, IFN-γ, IL-6, TNF-α) showed higher values in nonresponder patients only, with patients responding to treatment and controls not significantly differing among themselves (Figure 1).

Figure 2. Predicted Probability of Responding to Antidepressant Treatment, as a Function of the Principal Component Scores for the 5 Cytokines (IL-8, MCP1, IFN-γ, IL-6, and TNF-α) That Significantly Differed Between Responders and Nonresponders

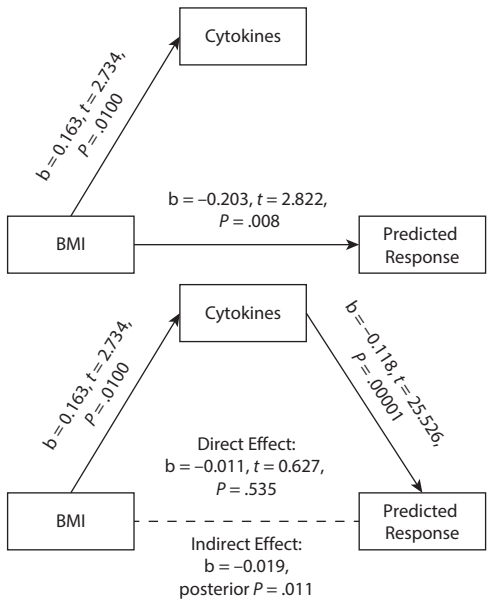


Abbreviations: IFN-γ = interferon γ, IL-6 = interleukin 6, IL-8 = interleukin 8, MCP1 = monocyte chemotactic protein 1, TNF-α = tumor necrosis factor α.

These 5 cytokines significantly correlated among themselves, and all showed a VIF > 1 (IL-8 = 23.75, MCP-1 = 3.22, IFN-γ = 46.42, IL-6 = 2.05, TNF-α = 18.48), yielding a mean VIF = 18.79. A PCA was then performed on the 5 factors and significantly identified a single component that explained 84.32% of variance (eigenvalue = 3.36, $Q^2 = 0.15$), and individual component scores were then extracted. A GLZM regression showed a significant negative effect of the factor scores on response to treatment (higher scores, worse response; parameter estimate = −0.692; Wald $W = 4.34$, $P = .037$) (Figure 2).

Body mass index correlated with current IDS-C ratings of depression (higher BMI, lower IDS-C score: $r = -0.412$, $P = .013$), and marginally associated with the observed response (Wald $W = 2.73$, $P = .098$). Body mass index significantly correlated with IFN-γ ($r = 0.431$, $P = .01$), IL-8 ($r = 0.462$, $P = .005$), TNF-α ($r = 0.373$, $P = .027$), and consequently with the PCA composite score for cytokines ($r = 0.430$, $P = .010$) and with the predicted probability to respond to treatment, as estimated by considering cytokine effects ($r = -0.441$, $P = .008$; Figure 2). The mediation analysis showed that this latter effect was indirect and fully mediated by cytokines (Figure 3): when both BMI and the cytokine composite score were considered together, the direct effect of BMI on predicted response disappeared, and the indirect effect was significant (b = −0.0192 with SE = 0.0084; 95% confidence limits, −0.0327 and −0.0001; partial posterior $P = .011$; effect size $\kappa^2 = 0.69$).

Figure 3. Mediation Model for the Effects of BMI on the Probability to Respond to Antidepressant Chronotherapeutics, as Estimated When Considering the Effects of the PCA Composite Scores for Cytokines[a] (see Figure 2)



[a]When considered alone, BMI shows a positive effect on cytokines and a negative effect on the probability to respond to treatment. This latter effect is fully mediated by cytokines and vanishes when the highly significant effect of cytokines (PCA component score for the 5 cytokines discriminating responders from nonresponders) is introduced into the model.

Abbreviations: b = regression coefficient, BMI = body mass index, PCA = principal component analysis.

Levels of cytokines did not significantly correlate with lithium levels in plasma and in erythrocytes, did not differ according to sex and ongoing lithium treatment, and did not correlate with CTQ scores.

During the following month, 8 of 23 responders showed signs of relapse, were treated with combined LT and antidepressant drugs upon clinical need in order to maintain the benefits from chronotherapeutics, and were discharged with a combined lithium and antidepressant drug treatment. The other 15 responders stayed well on lithium alone. Baseline cytokines did not influence this early relapse rate.

DISCUSSION

This study is the first to investigate the predictive effect of a panel of immune regulatory compounds on the antidepressant response of TSD + LT treatment in bipolar depression. We observed that higher levels of a set of 5 highly intercorrelating compounds associated with worse response to antidepressant TSD + LT treatment. The 5 compounds—IL-8, MCP-1, IFN-γ, IL-6, and TNF-α—showed higher baseline values in nonresponders than in responders and significantly predicted response. These results replicate and extend our previous findings of the predictive power of IL-6 in bipolar disorder (see Introduction).

Our study does not allow us to draw conclusive inferences about the pathological process that sustained the proinflammatory state. A principal component analysis identified a single factor explaining >80% of the mathematical variance of the 5 compounds, thus suggesting that the enhanced levels of these cytokines could be part of the same process. Interestingly, 3 of the compounds are reflecting the M1-like proinflammatory state of monocytes/macrophages (IL-8, IL-6, and TNF-α), 1 is a T cell factor driving the M1-like proinflammatory state of macrophages (IFN-γ), and 1 is strongly produced when monocytes diapedese transendothelially to the tissues (MCP-1 or CCL2 [chemokine ligand 2]). It can thus be hypothesized that the process involved in nonresponsiveness to TSD + LT could be the tissue infiltration of monocytes and their conversion to M1 proinflammatory macrophages. In particular, MCP-1 is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages into peripheral tissues, including the brain.[59] This finding is consistent with current hypotheses about neuroinflammation in bipolar disorder, which propose a major role for the activation of brain mononuclear phagocytes (microglia and macrophages) and local invasion of circulating immune cells,[60] with an aberrant expression of M1-like inflammatory genes consistently reported in monocytes of patients with bipolar disorder[61,62] and with in vivo detection of activated microglia at PET scans.[63]

Peripheral cytokines can enter the brain by volume diffusion or via active cytokine transporters at the blood-brain barrier,[64] and the M1-like cytokines that discriminated nonresponders could also exert their detrimental action by directly acting on neurotransmitters involved in the antidepressant response. Acting through the signal transducer and activator of transcription 3 (STAT3) canonical inflammatory signaling pathway, IL-6 down-regulates messenger RNA and protein levels of the serotonin (5-HT) transporter (SERT),[65] a phenotype that has been consistently associated with poor response to several antidepressant treatments[66] including TSD + LT.[35,67] Interleukin 6 also attenuates the receptor signaling from 5-HT_{2A},[68] which is involved in antidepressant response.[69] Acting on p38 mitogen-activated protein kinase (MAPK), TNF-α stimulates 5-HT uptake by decreasing 5-HT Km and increasing SERT V_{max},[70] an effect which goes in the opposite direction to the antidepressant mechanisms of the selective 5-HT reuptake inhibitor drugs[69] and that can trigger despair-like behavior in mice.[71] Interleukin 8 and MCP-1 modulate the electrical activity of several neuronal populations, thus playing a role as neurotransmitters and neuromodulators, and influence the activity of neurotransmitters involved in bipolar depression, such as dopamine.[72] MCP-1 hyperpolarizes 5-HT raphe neurons in mouse midbrain slices, thus probably reducing the 5-HT tone in projection areas.[73]

A brain proinflammatory state can also perturb the neuroimmune mechanisms that influence neuronal activity, as well as cortical synaptic plasticity,[74] which is a key correlate of antidepressant response to several treatments, including TSD+LT.[75] It can also exert detrimental effect on glial functions: in a previous study, we showed that levels of circulating IL-8, IFN-γ, and TNF-α are associated with signs of disrupted white matter microstructure in patients with bipolar disorder,[76] a phenotype associated with poor response to antidepressants including TSD.[77]

Signs of inflammation have been observed both in vivo[63] and postmortem[78] in the brain of patients with bipolar disorder, but peripheral factors can also contribute to raise the levels of circulating cytokines. In the obese adipose tissue, M1–polarized macrophages can secrete inflammatory cytokines,[79] which could contribute to metabolic dysregulation and insulin resistance,[79,80] thus hampering treatment response in bipolar disorder.[81] Here we showed, for the first time, that BMI can indirectly hamper antidepressant response by increasing the levels of proinflammatory cytokines that discriminate responders from nonresponders. The effect of BMI revealed only when considering cytokines. The weak, nonsignificant direct effects of BMI are in agreement with previous negative literature reports.[82,83] The association of BMI with increased cytokines has also been observed in other psychiatric conditions, such as schizophrenia.[84]

All these mechanisms could then concur to hamper response in patients with high M1-like proinflammatory cytokine levels.

Here we must limit inferences about differences between bipolar disorder and healthy controls, because of the age difference between the 2 groups. However, our observation of higher peripheral levels of ICAM-1 associated with the bipolar disorder diagnosis confirms 2 previous reports.[85,86] Moreover, ICAM-1, playing key roles in leukocyte–endothelial cell adhesion and transmigration from microvessels, has been found to be higher in postmortem brain tissue of patients affected both by bipolar disorder [63] and MDD,[87] thus suggesting the presence of brain inflammation associated with these conditions. Future studies, also taking into account other inflammatory markers associated with mood disorders,[88] will clarify the relative role of single factors as state and trait biomarkers of illness.

Limitations, however, do not impact the main finding of a worse M1-like proinflammatory state in nonresponders versus responders. Bipolar depression is a difficult-to-treat condition, with extremely low success rates of antidepressants,[89] and represents the predominant abnormal mood state in bipolar disorder.[90] About 20% of patients with bipolar disorder die by suicide,[91] and preliminary data suggest that an aberrant activation of the monocyte/macrophage system could influence this behavior.[92] Still preliminary, but consistent, evidence suggests that Cox-2 inhibitors potentiate antidepressants in MDD,[93] and in 1 trial[94] they showed antidepressant effects in bipolar disorder patients during depressive or mixed episodes. Our study raises the possibility that stratification of patients with bipolar depression on the basis of immune signatures could help to select a specific treatment-resistant subgroup that could benefit from the therapeutic targeting of the activated inflammatory response system.

Submitted: October 28, 2016; accepted January 17, 2017.

Published online: September 12, 2017.

Potential conflicts of interest: Authors have no personal direct support or financial affiliations to disclose.

Funding/support: This study was supported by the European Union EU-FP7-HEALTH-F2-2008-222963 MOODINFLAME and EU-FP7-PEOPLE-2009-IAPP PSYCH-AID projects.

Role of the sponsor: The funding agency approved the general design and conduct of the study but had no role in collection, management, analysis, or interpretation of the data or in preparation, review, or approval of the manuscript.

REFERENCES

1. Dantzer R. Depression and inflammation: an intricate relationship. *Biol Psychiatry*. 2012;71(1):4–5.
2. Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. *Neurosci Biobehav Rev*. 2012;36(2):764–785.
3. Bergink V, Gibney SM, Drexhage HA. Autoimmunity, inflammation, and psychosis: a search for peripheral markers. *Biol Psychiatry*. 2014;75(4):324–331.
4. Anderson G, Maes M. Bipolar disorder: role of immune-inflammatory cytokines, oxidative and nitrosative stress and tryptophan catabolites. *Curr Psychiatry Rep*. 2015;17(2):8.
5. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol*. 2006;27(1):24–31.
6. Lanquillon S, Krieg JC, Bening-Abu-Shach U, et al. Cytokine production and treatment response in major depressive disorder. *Neuropsychopharmacology*. 2000;22(4):370–379.
7. Sluzewska A, Sobieska M, Rybakowski JK. Changes in acute-phase proteins during lithium potentiation of antidepressants in refractory depression. *Neuropsychobiology*. 1997;35(3):123–127.
8. Maes M, Bosmans E, De Jongh R, et al. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine*. 1997;9(11):853–858.
9. Yoshimura R, Hori H, Ikenouchi-Sugita A, et al. Higher plasma interleukin-6 (IL-6) level is associated with SSRI- or SNRI-refractory depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2009;33(4):722–726.
10. Eller T, Vasar V, Shlik J, et al. Pro-inflammatory cytokines and treatment response to escitalopram in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008;32(2):445–450.
11. Rethorst CD, Toups MS, Greer TL, et al. Pro-inflammatory cytokines as predictors of antidepressant effects of exercise in major depressive disorder. *Mol Psychiatry*. 2013;18(10):1119–1124.
12. Mikova O, Yakimova R, Bosmans E, et al. Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis. *Eur Neuropsychopharmacol*. 2001;11(3):203–208.
13. Manoharan A, Rajkumar RP, Shewade DG, et al. Evaluation of interleukin-6 and serotonin as biomarkers to predict response to fluoxetine. *Hum Psychopharmacol*. 2016;31(3):178–184.
14. Hannestad J, DellaGioia N, Bloch M. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology*. 2011;36(12):2452–2459.
15. Benedetti F, Lucca A, Brambilla F, et al. Interleukine-6 serum levels correlate with response to antidepressant sleep deprivation and sleep phase advance. *Prog Neuropsychopharmacol Biol Psychiatry*. 2002;26(6):1167–1170.
16. Benedetti F, Poletti S, Hoogenboezem TA, et al. Stem Cell Factor (SCF) is a putative biomarker of antidepressant response. *J Neuroimmune Pharmacol*. 2016;11(2):248–258.
17. Goldstein BI, Kemp DE, Soczynska JK, et al. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. *J Clin Psychiatry*. 2009;70(8):1078–1090.
18. Becking K, Boschloo L, Vogelzangs N, et al. The association between immune activation and manic symptoms in patients with a depressive disorder. *Transl Psychiatry*. 2013;3:e314.
19. Bhattacharjee Y. Psychiatric research: is internal timing key to mental health? *Science*. 2007;317(5844):1488–1490.
20. Wirz-Justice A, Benedetti F, Terman M. *Chronotherapeutics for Affective Disorders. A Clinician's Manual for Light and Wake Therapy*. Basel, Switzerland: Karger; 2009.
21. Thase ME. Antidepressant combinations: widely used, but far from empirically validated. *Can J Psychiatry*. 2011;56(6):317–323.
22. Frye MA, Helleman G, McElroy SL, et al. Correlates of treatment-emergent mania associated with antidepressant treatment in bipolar depression. *Am J Psychiatry*. 2009;166(2):164–172.
23. Gao K, Kemp DE, Ganocy SJ, et al. Treatment-emergent mania/hypomania during antidepressant monotherapy in patients with rapid cycling bipolar disorder. *Bipolar Disord*. 2008;10(8):907–915.
24. Altshuler L, Suppes T, Black D, et al. Impact of antidepressant discontinuation after acute bipolar depression remission on rates of depressive relapse at 1-year follow-up. *Am J Psychiatry*. 2003;160(7):1252–1262.
25. Post RM, Altshuler LL, Frye MA, et al. Complexity of pharmacologic treatment required for sustained improvement in outpatients with bipolar disorder. *J Clin Psychiatry*. 2010;71(9):1176–1186, quiz 1252–1253.
26. Benedetti F, Colombo C. Sleep deprivation in mood disorders. *Neuropsychobiology*. 2011;64(3):141–151.
27. Benedetti F, Barbini B, Colombo C, et al. Chronotherapeutics in a psychiatric ward. *Sleep Med Rev*. 2007;11(6):509–522.
28. Wirz-Justice A, Benedetti F, Berger M, et al. Chronotherapeutics (light and wake therapy) in affective disorders. *Psychol Med*. 2005;35(7):939–944.
29. Benedetti F, Dallaspezia S, Fulgosi MC, et al. Phase advance is an actimetric correlate of antidepressant response to sleep deprivation and light therapy in bipolar depression. *Chronobiol Int*. 2007;24(5):921–937.
30. Benedetti F, Barbini B, Fulgosi MC, et al. Combined total sleep deprivation and light therapy in the treatment of drug-resistant bipolar depression: acute response and long-term remission rates. *J Clin Psychiatry*. 2005;66(12):1535–1540.
31. Colombo C, Lucca A, Benedetti F, et al. Total sleep deprivation combined with lithium and light therapy in the treatment of bipolar depression: replication of main effects and interaction. *Psychiatry Res*. 2000;95(1):43–53.
32. Benedetti F, Riccaboni R, Locatelli C, et al. Rapid treatment response of suicidal symptoms to lithium, sleep deprivation, and light therapy (chronotherapeutics) in drug-resistant bipolar depression. *J Clin Psychiatry*. 2014;75(2):133–140.
33. Benedetti F, Barbini B, Bernasconi A, et al. Acute antidepressant response to sleep deprivation combined with light therapy is influenced by the catechol-O-methyltransferase Val(108/158)Met polymorphism. *J Affect Disord*. 2010;121(1–2):68–72.
34. Benedetti F, Dallaspezia S, Lorenzi C, et al. Gene-gene interaction of glycogen synthase kinase 3-β and serotonin transporter on human antidepressant response to sleep deprivation. *J Affect Disord*. 2012;136(3):514–519.
35. Benedetti F, Bernasconi A, Blasi V, et al. Neural and genetic correlates of antidepressant response to sleep deprivation: a functional magnetic resonance imaging study of moral valence decision in bipolar depression. *Arch Gen Psychiatry*. 2007;64(2):179–187.
36. Benedetti F, Smeraldi E. Neuroimaging and genetics of antidepressant response to sleep deprivation: implications for drug development. *Curr Pharm Des*. 2009;15(22):2637–2649.
37. Ebert D, Berger M. Neurobiological similarities in antidepressant sleep deprivation and psychostimulant use: a psychostimulant theory of antidepressant sleep deprivation. *Psychopharmacology (Berl)*. 1998;140(1):1–10.
38. Gillin JC, Buchsbaum M, Wu J, et al. Sleep deprivation as a model experimental antidepressant treatment: findings from functional brain imaging. *Depress Anxiety*. 2001;14(1):37–49.
39. Zarate CA Jr, Mathews DC, Furey ML. Human biomarkers of rapid antidepressant effects. *Biol Psychiatry*. 2013;73(12):1142–1155.
40. Thase ME, Rush AJ. When at first you don't succeed: sequential strategies for antidepressant nonresponders. *J Clin Psychiatry*. 1997;58(suppl 13):23–29.
41. Benedetti F, Barbini B, Bernasconi A, et al. Lithium overcomes the influence of 5-HTTLPR gene polymorphism on antidepressant response to sleep deprivation. *J Clin Psychopharmacol*. 2008;28(2):249–251.
42. Rush AJ, Gullion CM, Basco MR, et al. The Inventory of Depressive Symptomatology (IDS): psychometric properties. *Psychol Med*. 1996;26(3):477–486.
43. Rush AJ, Kraemer HC, Sackeim HA, et al; ACNP Task Force. Report by the ACNP Task Force on response and remission in major depressive disorder. *Neuropsychopharmacology*. 2006;31(9):1841–1853.
44. Janiri D, Sani G, Danese E, et al. Childhood traumatic experiences of patients with bipolar disorder type I and type II. *J Affect Disord*. 2015;175:92–97.
45. Schmohl M, Schneiderhan-Marra N, Baur N, et al. Characterization of immunologically active drugs in a novel organotypic co-culture model of the human gut and whole blood. *Int Immunopharmacol*. 2012;14(4):722–728.
46. McCulloch CE, Searle SR, Neuhaus JM. *Generalized, Linear, and Mixed Models*. 2nd ed. New York: John Wiley & Sons; 2008.
47. Timm N, Kim K. *Univariate and Multivariate General Linear Models: Theory and Applications with SAS*. 2nd ed. Berlin, Heidelberg: Springer; 2006.
48. Hill T, Lewicki P. Statistics: methods and

applications. *A Comprehensive Reference for Science, Industry, and Data Mining*, Chapter 18. Tulsa, OK: General Linear Models, StatSoft; 2006:245–276.
49. Benjamini Y, Krieger A, Yekutieli D. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika*. 2006;93:491–507.
50. Bowerman BL, O'Connell RT. *Linear Statistical Models: An Applied Approach*. 2nd ed. Pacific Grove, CA: Duxbury; 1990.
51. Lafi SQ, Kaneene JB. An explanation of the use of principal components analysis to detect and correct for multicollinearity. *Prev Vet Med*. 1992;13:261–275.
52. Dormann CF, Elith J, Bacher S, et al. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*. 2013;36:27–46.
53. Field A. *Discovering Statistics Using IBM SPSS Statistics*. 4th ed. Thousand Oaks, CA: SAGE; 2013.
54. McCullagh P, Nelder JA. *Generalized linear models*. 2nd ed. New York, NY: Chapman & Hall; 1989.
55. Ferrante AW Jr. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. *J Intern Med*. 2007;262(4):408–414.
56. Hayes AF. *Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach*. New York, NY: Guilford Press; 2013.
57. Preacher KJ, Kelley K. Effect size measures for mediation models: quantitative strategies for communicating indirect effects. *Psychol Methods*. 2011;16(2):93–115.
58. Biesanz JC, Falk CF, Savalei V. Assessing mediational models: testing and interval estimation for indirect effects. *Multivariate Behav Res*. 2010;45(4):661–701.
59. Deshmane SL, Kremlev S, Amini S, et al. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res*. 2009;29(6):313–326.
60. Beumer W, Gibney SM, Drexhage RC, et al. The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes. *J Leukoc Biol*. 2012;92(5):959–975.
61. Padmos RC, Hillegers MH, Knijff EM, et al. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. *Arch Gen Psychiatry*. 2008;65(4):395–407.
62. Padmos RC, Van Baal GC, Vonk R, et al. Genetic and environmental influences on pro-inflammatory monocytes in bipolar disorder: a twin study. *Arch Gen Psychiatry*. 2009;66(9):957–965.
63. Haarman BC, Riemersma-Van der Lek RF, de Groot JC, et al. Neuroinflammation in bipolar disorder - A [(11)C]-(R)-PK11195 positron emission tomography study. *Brain Behav Immun*. 2014;40:219–225.
64. Dantzer R, O'Connor JC, Freund GG, et al. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008;9(1):46–56.
65. Kong E, Sucic S, Monje FJ, et al. STAT3 controls IL6-dependent regulation of serotonin transporter function and depression-like behavior. *Sci Rep*. 2015;5:9009.
66. Serretti A, Benedetti F, Zanardi R, et al. The influence of Serotonin Transporter Promoter Polymorphism (SERTPR) and other polymorphisms of the serotonin pathway on the efficacy of antidepressant treatments. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005;29(6):1074–1084.
67. Benedetti F, Colombo C, Serretti A, et al. Antidepressant effects of light therapy combined with sleep deprivation are influenced by a functional polymorphism within the promoter of the serotonin transporter gene. *Biol Psychiatry*. 2003;54(7):687–692.
68. Donegan JJ, Patton MS, Chavera TS, et al. Interleukin-6 attenuates serotonin 2a receptor signaling by activating the JAK-STAT pathway. *Mol Pharmacol*. 2015;87(3):492–500.
69. Artigas F, Nutt DJ, Shelton R. Mechanism of action of antidepressants. *Psychopharmacol Bull*. 2002;36(suppl 2):123–132.
70. Zhu CB, Blakely RD, Hewlett WA. The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. *Neuropsychopharmacology*. 2006;31(10):2121–2131.
71. Zhu CB, Lindler KM, Owens AW, et al. Interleukin-1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. *Neuropsychopharmacology*. 2010;35(13):2510–2520.
72. Rostène W, Dansereau MA, Godefroy D, et al. Neurochemokines: a menage a trois providing new insights on the functions of chemokines in the central nervous system. *J Neurochem*. 2011;118(5):680–694.
73. Cazareth J, Guyon A, Heurteaux C, et al. Molecular and cellular neuroinflammatory status of mouse brain after systemic lipopolysaccharide challenge: importance of CCR2/CCL2 signaling. *J Neuroinflammation*. 2014;11:132.
74. Wohleb ES, Franklin T, Iwata M, et al. Integrating neuroimmune systems in the neurobiology of depression. *Nat Rev Neurosci*. 2016;17(8):497–511.
75. Canali P, Sferrazza Papa G, Casali AG, et al. Changes of cortical excitability as markers of antidepressant response in bipolar depression: preliminary data obtained by combining transcranial magnetic stimulation (TMS) and electroencephalography (EEG). *Bipolar Disord*. 2014;16(8):809–819.
76. Benedetti F, Poletti S, Hoogenboezem TA, et al. Inflammatory cytokines influence measures of white matter integrity in Bipolar Disorder. *J Affect Disord*. 2016;202:1–9.
77. Bollettini I, Poletti S, Locatelli C, et al. Disruption of white matter integrity marks poor antidepressant response in bipolar disorder. *J Affect Disord*. 2015;174:233–240.
78. Thomas AJ, Davis S, Ferrier IN, et al. Elevation of cell adhesion molecule immunoreactivity in the anterior cingulate cortex in bipolar disorder. *Biol Psychiatry*. 2004;55(6):652–655.
79. Chatzigeorgiou A, Chavakis T. Immune cells and metabolism. *Handbook Exp Pharmacol*. 2016;233:221–249.
80. Baldeón R L, Weigelt K, de Wit H, et al. Decreased serum level of miR-146a as sign of chronic inflammation in type 2 diabetic patients. *PLoS One*. 2014;9(12):e115209.
81. Calkin CV, Ruzickova M, Uher R, et al. Insulin resistance and outcome in bipolar disorder. *Br J Psychiatry*. 2015;206(1):52–57.
82. Mansoor B, Rengasamy M, Hilton R, et al. The bidirectional relationship between body mass index and treatment outcome in adolescents with treatment-resistant depression. *J Child Adolesc Psychopharmacol*. 2013;23(7):458–467.
83. Woo YS, Seo HJ, McIntyre RS, et al. Obesity and its potential effects on antidepressant treatment outcomes in patients with depressive disorders: a literature review. *Int J Mol Sci*. 2016;17(1):E80.
84. Beumer W, Drexhage RC, De Wit H, et al. Increased level of serum cytokines, chemokines and adipokines in patients with schizophrenia is associated with disease and metabolic syndrome. *Psychoneuroendocrinology*. 2012;37(12):1901–1911.
85. Pantović-Stefanović M, Petronijević N, Dunjić-Kostić B, et al. sVCAM-1, sICAM-1, TNF-α and IL-6 levels in bipolar disorder type I: acute, longitudinal and therapeutic implications [published online ahead of print December 14, 2016]. *World J Biol Psychiatry*.
86. Turan C, Kesebir S, Süner O. Are ICAM, VCAM and E-selectin levels different in first manic episode and subsequent remission? *J Affect Disord*. 2014;163:76–80.
87. Thomas AJ, Ferrier IN, Kalaria RN, et al. Elevation in late-life depression of intercellular adhesion molecule-1 expression in the dorsolateral prefrontal cortex. *Am J Psychiatry*. 2000;157(10):1682–1684.
88. Haapakoski R, Mathieu J, Ebmeier KP, et al. Cumulative meta-analysis of interleukins 6 and 1β, tumor necrosis factor α and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun*. 2015;49:206–215.
89. Post RM, Leverich GS, Altshuler LL, et al. Differential clinical characteristics, medication usage, and treatment response of bipolar disorder in the US versus The Netherlands and Germany. *Int Clin Psychopharmacol*. 2011;26(2):96–106.
90. Kupka RW, Altshuler LL, Nolen WA, et al. Three times more days depressed than manic or hypomanic in both bipolar I and bipolar II disorder. *Bipolar Disord*. 2007;9(5):531–535.
91. Leverich GS, Altshuler LL, Frye MA, et al. Factors associated with suicide attempts in 648 patients with bipolar disorder in the Stanley Foundation Bipolar Network. *J Clin Psychiatry*. 2003;64(5):506–515.
92. Steiner J, Gos T, Bogerts B, et al. Possible impact of microglial cells and the monocyte-macrophage system on suicidal behavior. *CNS Neurol Disord Drug Targets*. 2013;12(7):971–979.
93. Andrade C. Antidepressant augmentation with anti-inflammatory agents. *J Clin Psychiatry*. 2014;75(9):975–977.
94. Nery FG, Monkul ES, Hatch JP, et al. Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study. *Hum Psychopharmacol*. 2008;23(2):87–94.