



Low serum IL-10 concentrations and loss of regulatory association between IL-6 and IL-10 in adults with major depression

Firdaus S. Dhabhar^{a,b,*,1}, Heather M. Burke^{c,1}, Elissa S. Epel^c, Synthia H. Mellon^d, Rebecca Rosser^e, Victor I. Reus^e, Owen M. Wolkowitz^e

^a Department of Psychiatry and Behavioral Sciences, Stanford University, School of Medicine, USA

^b Stanford Institute for Immunity, Transplantation and Infection, Stanford University School of Medicine, USA

^c Health Psychology Program, Department of Psychiatry, University of California, San Francisco, School of Medicine, USA

^d Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, School of Medicine, USA

^e Department of Psychiatry, Langley Porter Psychiatric Institute, University of California, San Francisco, School of Medicine, USA

ARTICLE INFO

Article history:

Received 10 March 2009

Received in revised form 28 May 2009

Accepted 29 May 2009

Keywords:

Depression

Cytokines

Inflammation

IL-6

IL-10

Immune regulation

Mood disorders

ABSTRACT

Elevated circulating pro-inflammatory cytokines are associated with symptoms of depression, and disorders involving chronic inflammation are often co-morbid with major depression. Since healthy immune regulation is accomplished through counter-balancing effects of pro- and anti-inflammatory cytokines, we hypothesized that depressed subjects (compared to controls) would express lower concentrations of the anti-inflammatory/immunoregulatory cytokine interleukin (IL)-10, and a higher IL-6/IL-10 ratio. We also examined the possibility that depressed subjects may exhibit a deficiency in the regulatory loop involving IL-6 induced secretion of IL-10. Therefore, we hypothesized that circulating IL-6 and IL-10 would be positively correlated in controls, while the correlation would be weaker in depressed subjects. Resting state serum cytokine concentrations were quantified in 12 unmedicated depressed subjects, and 11 age, gender, and ethnicity-matched controls. Depressed subjects showed significantly lower IL-10 ($p = 0.03$, Cohen's $d = -0.96$), non-significantly higher IL-6, and significantly higher IL-6/IL-10 ratios ($p = 0.05$, Cohen's $d = 0.50$). Across all participants, higher scores on the self-rated Inventory of Depressive Symptoms were associated with lower IL-10 ($r(21) = -0.57$, $p = 0.005$) and non-significantly higher IL-6/IL-10 ratios ($r(21) = 0.38$, $p = 0.07$), but not related to IL-6 concentrations. As hypothesized, IL-6 and IL-10 concentrations were strongly and positively correlated in controls ($r(9) = 0.81$, $p = 0.003$), but were completely dissociated in depressed subjects ($r(10) = 0.01$, $p = 0.98$). These results suggest that lower IL-10 levels, a higher IL-6/IL-10 ratio, and the apparent absence of a counter-balancing, immunoregulatory increase in IL-10 in response to elevated IL-6 concentrations contribute to the pro-inflammatory physiological milieu that is known to be associated with major depression. Therefore, reduced induction/availability of IL-10, that would normally inhibit pro-inflammatory cytokine actions and resolve inflammation, may contribute to the depressogenic as well as the inflammatory disease-promoting effects of chronic, low-level elevations in pro-inflammatory cytokines.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Major depression is one of the most common psychiatric disorders (Schatzberg, 2005), and has been ranked by some estimates as the fourth leading cause of disease burden worldwide (Mathers and Loncar, 2006). In addition to the significant psychological and physical morbidity intrinsic to this disorder, other consequences include increased risk of mortality through cardiovascular disease (Wulsin et al., 1999) and cancer (Goodwin et al., 2004;

Hjerl et al., 2003; Onitilo et al., 2006; Stommel et al., 2002). It has been suggested that pro-inflammatory cytokines may cause (or contribute to) depression in susceptible individuals during disorders involving chronic inflammation (Dantzer et al., 2008b; Miller et al., 2009) and studies have shown that pro-inflammatory cytokines are often associated with the development of depression (Capuron and Dantzer, 2003; Howren et al., 2009; Irwin and Miller, 2007; Kiecolt-Glaser and Glaser, 2002; Maes, 1995; Miller et al., 2009, 2002; Pace et al., 2006; Pollak and Yirmiya, 2002; Raison et al., 2006). Pre-clinical studies have shown that animals exposed to increased levels of endogenous or exogenously-administered pro-inflammatory cytokines exhibit a constellation of depression-like symptoms that have been collectively referred to as "sickness behavior," (Dantzer et al., 2008b; Larson and Dunn, 2001; Pollak

* Corresponding author. Address: Stanford University, 300 Pasteur Drive, MC 5135, Stanford, CA 94305, USA. Tel.: +1 650 736 8565.

E-mail address: dhabhar@gmail.com (F.S. Dhabhar).

¹ FSD and HMB contributed equally to this work.

and Yirmiya, 2002; Watkins and Maier, 2005). Clinical support for a role of pro-inflammatory cytokines in major depression comes from case-controlled studies demonstrating elevated circulating interleukin (IL)-6 concentrations in depressed adults (Maes et al., 1997; Miller et al., 2002; O'Brien et al., 2007; Pace et al., 2006), observational clinical studies finding positive correlations between depressive symptoms and circulating IL-6 concentrations (Glaser et al., 2003; Penninx et al., 2003; Sjogren et al., 2006; Suarez, 2003), experimental human studies demonstrating development of depression following infusion of cytokines such as IFN- α (Capuron and Miller, 2004; Musselman et al., 2001; Wichers et al., 2007), human studies demonstrating anti-depressant effects of etanercept (a TNF- α inhibitor) in patients with psoriasis (Tyring et al., 2006), and antidepressant treatment studies demonstrating decreases in pro-inflammatory cytokines following recovery from major depression (Basterzi et al., 2005; Castanon et al., 2002; Kenis and Maes, 2002; Kubera et al., 2001; O'Brien et al., 2007). It has been suggested that common genetic vulnerability involving genes within the inflammatory and serotonin pathways may contribute to depression and coronary artery disease (McCaffery et al., 2006), and a recent study has shown that individuals expressing the "low IL-6" synthesizing polymorphism of the IL-6 promoter showed significantly fewer depressive symptoms following IFN- α treatment (Bull et al., 2008).

It is important to recognize that in the absence of chronic infection, cancer, or immuno-deficiency disease, modest elevations in anti-inflammatory cytokines may exert salubrious or health-protective effects by mediating the resolution of inflammatory processes and preventing or antagonizing the damaging effects of prolonged inflammation. In contrast to pro-inflammatory cytokines such as IL-6, considerably less attention has been focused on the potential role of anti-inflammatory/immunomodulatory cytokines such as IL-10 in depression. In healthy individuals, there is a regulated balance between pro- and anti-inflammatory cytokines: For example, IL-6 mediates the early phase of the inflammatory process, and then induces the release of IL-10 that exerts immuno-regulatory effects and resolves inflammation (Daftarian et al., 1996; Fang et al., 2008; Ogawa et al., 2008). The net result of such regulatory relationships is that the immune system can quickly respond to challenge and subsequently return to homeostasis once the challenge has ended. Short-term inflammatory reactions in response to immune challenges such as wounding or infection are adaptive and essential for survival, but chronic inflammation is harmful. Interestingly, depression is associated with numerous disorders that are thought to involve chronic inflammation (Dantzer et al., 2008a; Evans et al., 2005; Kiecolt-Glaser and Glaser, 2002). These include cardiovascular disease (Frasure-Smith et al., 2007; McCaffery et al., 2006; Musselman and Nemeroff, 2000; Whooley et al., 2007), obesity (Onyike et al., 2003), rheumatoid arthritis (Bruce, 2008; Zautra et al., 2004), and multiple sclerosis (Triantafyllou et al., 2008).

An important and relatively under-appreciated mechanism for the link between depression and inflammatory disorders may be a disruption of the immuno-regulatory balance between pro- and anti-inflammatory cytokines. The cytokine balance can be shifted towards a pro-inflammatory milieu due to elevated concentrations of pro-inflammatory cytokines, such as IL-6, lowered concentrations of anti-inflammatory/immunomodulatory cytokines, such as IL-10, or a combination of the two. Therefore, in addition to their absolute concentrations, the relative concentrations of pro- to anti-inflammatory cytokines may provide a useful index of the net inflammatory milieu and of immune dysregulation. With this in mind, we hypothesized that relative to controls, depressed subjects would exhibit lower IL-10 concentrations, higher circulating IL-6 concentrations, and a higher IL-6/IL-10 ratio. Furthermore, we hypothesized that across the entire sample of participants, higher

levels of depressive symptoms would be associated with lower IL-10 and higher IL-6 concentrations, as well as higher IL-6/IL-10 ratios. Importantly, we also examined the possibility that depressed subjects may have a deficiency in the feed-forward loop between IL-6 and IL-10. This would result in insufficient secretion of IL-10 (and perhaps other anti-inflammatory cytokines) in response to stimulation during inflammatory reactions driven by IL-6 (and other pro-inflammatory cytokines). Reduced IL-10 levels would disrupt/prevent the resolution of inflammation, impair the inhibition of other actions of pro-inflammatory cytokines on non-inflamed tissues, and allow detrimental progression from acute to chronic inflammation. Keeping the importance of this IL-6 and IL-10 feed-forward loop in mind, we hypothesized that circulating IL-6 and IL-10 concentrations would be positively correlated in control subjects whereas the correlation would be weaker in depressed subjects. This manuscript presents the results that were obtained after testing these hypotheses and discusses the potential implications of our findings.

2. Methods

2.1. Sample characteristics

Unmedicated depressed and healthy control subjects were recruited from the San Francisco Bay Area community and Langley Porter Psychiatric Institute at the University of California San Francisco (UCSF). Inclusion criteria for the depressed subjects included: (1) DSM-IV criteria for unipolar major depression based on the Structured Clinical Interview for DSM Disorders – non-patient version (SCID-I/NP; (First et al., 2002)), (2) 17-item Hamilton Depression Rating Scale scores ≥ 17 (HAMD; (Hamilton, 1960)), (3) medically healthy, (4) clinical labs (complete blood count, electrolytes, liver, thyroid and renal function tests) with no clinically significant abnormalities, (5) negative urine toxicology (drugs of abuse) screen, (6) age greater than 18 but less than 70 years, (7) English speaking, and (8) ability to provide informed consent. Exclusion criteria included: (1) pregnancy, (2) medical illnesses (e.g. autoimmune diseases, diabetes, HIV, endocrine disorders, hepatitis, cancer, or chronic infections) or medications (e.g. steroid medications, antioxidants, corticosteroids (oral, injected, inhaled and/or topical), birth control medications, immunotherapy, antibiotics) that could affect cytokine concentrations, (3) febrile illness (temperature $>99^{\circ}\text{F}$) or elevated WBC counts (WBC $>10,000$), (4) immunizations within 4 weeks of the blood draw, (5) psychotropic medication use (including antidepressants, mood stabilizers, anti-anxiety medications or antipsychotics) within previous 6 weeks (although low dose, short-acting sedative-hypnotic medication was allowed as needed at bedtime (<3 times per week) up to 3 days prior to the blood draw), and (6) meeting DSM-IV criteria for psychotic, bipolar, or post-traumatic stress disorder, or for drug or alcohol abuse within the past 6 months. Healthy control subjects were individually matched to subjects who had major depression according to age (± 2 –3 years), gender and ethnicity, and had to meet all of the above criteria plus have no past or present DSM-IV Axis I disorder, as determined by the SCID-I/NP. One depressed subject did not have a matched control at the time of analyses, leaving a total of 12 depressed and 11 control subjects who were included in the study.

Detailed characteristics of the depressed and healthy control subjects are presented in Table 1. Results of chi-square analyses revealed no significant differences between depressed and healthy control subjects in marital status ($\chi^2(3) = 1.69, p = 0.64$) or education ($\chi^2(3) = 2.31, p = 0.51$). Since body mass index (BMI) scores were skewed, we used Mann–Whitney *U*-tests to compare BMI scores between groups. Depressed subjects had higher BMI scores than

Table 1
Sample characteristics by group.

Means (SD)	Controls (n = 11)	Depressed (n = 12)
Age (years)	38.00 (13.27)	38.42 (11.03)
Body mass index (kg/m ²) [*]	24.37 (3.61)	28.67 (5.95)
Inventory of Depressive Symptoms ^{**}	5.64 (5.28)	33.58 (10.07)
Hamilton Depression Rating Scale		20.58 (3.09)
Counts	<i>n</i>	<i>n</i>
Gender	5M: 6 F	5M: 7 F
<i>Marital status</i>		
Married/partnered	4	2
Divorced	2	3
Never married	5	7
<i>Race/ethnicity</i>		
White	8	8
Black	2	2
Asian/Pacific Islander	0	1
Other	1	1
<i>Education</i>		
Some college	4	4
College graduate	4	7
Advanced degree	3	1

^{*} $p < 0.05$.

^{**} $p < 0.0001$.

controls (Mann–Whitney $U = 31.00$, $z = -2.16$, $p = .03$, Cohen's $d = 0.86$). Depressive symptoms were assessed using the self-rated Inventory of Depressive Symptoms (IDS) (Rush et al., 1986) in all participants, and the HAMD in depressed participants only. Depressed participants had higher self-rated IDS scores than controls ($F(1, 22) = 67.55$, $p < 0.0001$), and the mean HAMD score in the depressed participants was 20.58 ± 3.09 (range: 17–26), reflecting a relatively mild-to-moderate degree of depressive symptom severity.

2.2. Procedures

This study was approved by the UCSF and Stanford Committees on Human Research and is registered with the NIH Clinical Trials database (NIH Clinical Trial Registry Number: NCT00285935). During the initial contact with subjects, the study was explained, and a preliminary brief diagnostic interview and review of entry criteria was conducted. After a complete description of the study to the subjects, written informed consent was obtained. Eligible, consenting subjects then underwent a SCID-I/NP interview by a trained clinical psychologist to evaluate the DSM-IV diagnosis and a complete psychiatric clinical interview and physical examination by a board-certified psychiatrist. The self-rated IDS questionnaire was completed by all participants, and the observer-rated HAMD was administered to the depressed subjects at this time by a psychologist to verify a sufficient degree of depressive symptom severity. Subsequently, subjects were admitted as outpatients to the UCSF Clinical Translational Science Institute at 8:00 am, having fasted (except for water) since 12:00 am the night before. Upon arrival, subjects had blood drawn via forearm venipuncture for clinical laboratory testing (e.g. glucose, electrolytes, CBC, liver and renal functions, thyroid function tests, lipid panel) to screen for illnesses, and they provided urine samples for drugs of abuse and pregnancy screening. The HAMD was re-administered at this time. After resting for 45 min in a recumbent position, subjects had additional blood drawn from the same indwelling intravenous catheter for assay of serum cytokines.

2.3. Cytokine assays

Samples were collected in 10 ml SST tubes (Becton Dickinson, Franklin Lakes, NJ). Serum was frozen and stored at -80°C . A high

sensitivity enzyme-linked immunosorbent assay was used to quantify IL-6 and IL-10 concentrations (R&D Systems, Minneapolis, MN). For IL-6, assay sensitivity is <0.1 pg/ml, and average intra- and inter-assay coefficients of variation are 7% and 8% respectively. For IL-10, assay sensitivity is 0.50 pg/ml, and average intra- and inter-assay coefficients of variation are 8% and 11% respectively.

2.4. Data analytic procedures

Mean serum IL-10 and IL-6 concentrations are presented in Fig. 1a and b, respectively. Cytokine concentrations that were below the limit-of-detection were set to near zero (0.001) to allow for computation of an IL-6/IL-10 ratio for each subject. Since the BMI and cytokine data were skewed, non-parametric, two-tailed tests ($\alpha = 0.05$) were used for the primary analyses. First, the effects of potential covariates such as age, gender and BMI on cytokine concentrations were tested using Spearman correlations across the whole range of participants. Second, serum concentrations of IL-10, IL-6, and IL-6/IL-10 ratios were rank ordered and Mann–Whitney U tests were used to compare the ranks between depressed subjects and controls. Next, Spearman correlation analyses were conducted to test associations between depressive symptoms (i.e. IDS scores) and cytokines across the pooled sample of participants. Finally, Spearman correlation analyses between IL-10 and IL-6 concentrations were conducted in depressed participants and controls separately. Follow-up partial correlation analyses controlling for potential effects of BMI on inter-cytokine correlations within groups were conducted using transformed BMI (log) and cytokine (square root) data to meet normality requirements for parametric tests. Because of the small sample size, effect sizes (Cohen's d for group comparisons, r for associations) were calculated for the primary analyses and classified into the following groups: “no effect,” “small effect” ($0.2 \leq d < 0.50$; $0.10 \leq r < 0.25$), “medium effect” ($0.50 \leq d < 0.80$; $0.25 \leq r < 0.40$), and “large effect” ($d \geq 0.80$; $r \geq .40$).

3. Results

Across the entire sample of age-matched subjects, there were no significant correlations between age and serum concentrations of IL-10 ($r(21) = -.34$, $p = .11$) or IL-6 ($r(21) = .29$, $p = .19$). Moreover, no gender differences in serum IL-10 (Mann–Whitney $U = 46.00$, $z = -1.18$, $p = .24$, Cohen's $d = -0.35$) or IL-6 concentrations (Mann–Whitney $U = 62.00$, $z = -0.19$, $p = .85$, Cohen's $d = 0.29$) were detected. Across the entire sample of participants, BMI scores were not significantly correlated with IL-10 (Spearman $r(21) = -0.22$, $p = 0.31$) or IL-6 ($r(21) = 0.12$, $p = 0.58$) concentrations. Since groups were matched for age, gender and ethnicity, these variables were not included as covariates in the analyses.

We first tested whether depressed subjects had lower concentrations of serum IL-10 and higher concentrations of IL-6 than age-, gender- and ethnicity-matched controls. Results revealed significantly lower serum IL-10 concentrations in depressed subjects (mean = 0.34 ± 0.11 , median = 0.29 pg/ml) compared to controls (mean = 0.83 ± 0.19 , median = 0.67 pg/ml), Mann–Whitney $U = 31.00$, $z = -2.16$, $p = 0.03$, Cohen's $d = -0.96$, large effect (Fig. 1a). Results also revealed non-significantly higher serum IL-6 concentrations in depressed subjects (mean = 1.09 ± 0.28 , median = 0.79 pg/ml) compared to controls (mean = 0.74 ± 0.12 , median = 0.70 pg/ml), Mann–Whitney $U = 59.00$, $z = -0.43$, $p = 0.67$, Cohen's $d = 0.47$ (small/medium effect) (Fig. 1b). Across the entire group of participants, higher scores on the IDS were strongly correlated with lower IL-10 concentrations (Spearman $r(21) = -0.57$, $p = 0.005$). However, in contrast to IL-10, IDS scores were not cor-

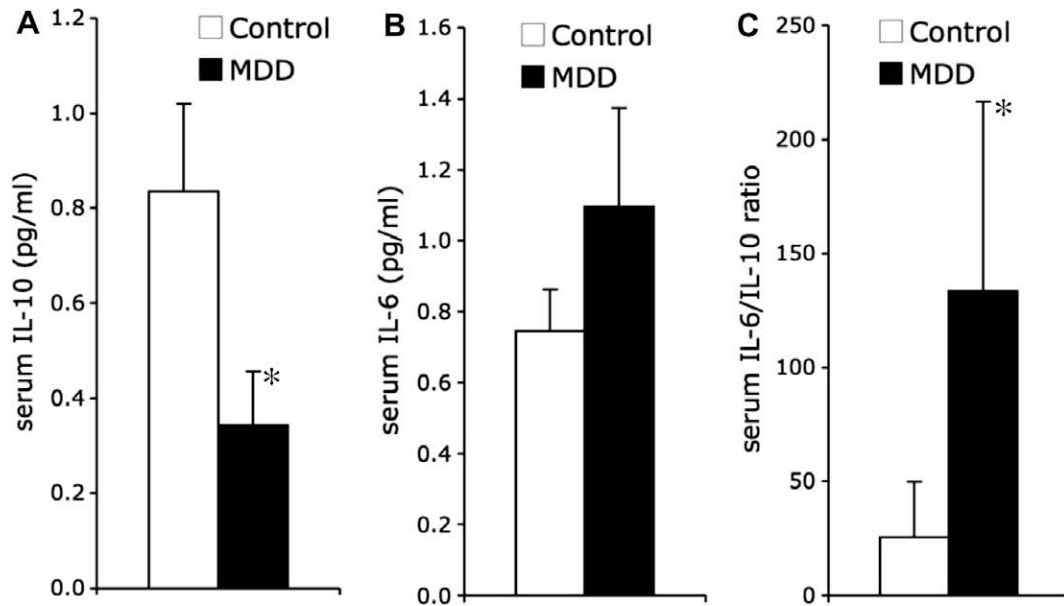


Fig. 1. Mean serum IL-10 (A) and IL-6 (B) concentrations, and IL-6/IL-10 ratio (C) in control versus depressed subjects. Compared to controls (white bars), depressed subjects (black bars) showed significantly lower circulating IL-10 concentrations (Mann–Whitney $U = 31.00$, $z = -2.16$, $p = 0.03$, Cohen's $d = -0.96$, large effect. Panel A); higher but statistically non-significant IL-6 concentrations (Mann–Whitney $U = 59.00$, $z = -0.43$, $p = 0.67$ Cohen's $d = 0.47$, small/medium effect. Panel B); and a significantly higher IL-6/IL-10 ratio (Mann–Whitney $U = 34.00$, $z = -1.97$, $p = .05$ Cohen's $d = 0.50$, medium effect. Panel C). Data are expressed as means + SEM.

related with IL-6 concentrations across the entire group of participants (Spearman $r(21) = -0.19$, $p = 0.38$).

To examine differences between groups in relative concentrations of these pro- and anti-inflammatory cytokines, we also compared the IL-6/IL-10 ratios between groups. Results revealed significantly higher IL-6/IL-10 ratios in depressed subjects (mean = 133.34 ± 83.28 , median = 2.93 pg/ml) compared to controls (mean = 25.49 ± 24.35 , median = 1.08 pg/ml), Mann–Whitney $U = 34.00$, $z = -1.97$, $p = .05$, Cohen's $d = 0.50$, medium effect), suggesting that the cytokine balance of depressed subjects was tilted in favor of pro-inflammation (Fig. 1c). Furthermore, there was a statistically non-significant trend for higher IL-6/IL-10 ratios to be associated with higher IDS scores across the entire sample of participants (Spearman $r(21) = 0.38$, $p = 0.07$).

To examine a potential deficiency in the feed-forward loop between IL-10 and IL-6 in depressed subjects, we examined the strength and nature (positive, negative, or flat) of the correlations between circulating IL-10 and IL-6 concentrations in depressed

subjects and controls. Serum IL-10 and IL-6 concentrations were strongly and positively correlated in controls ($r(9) = 0.81$, $p = 0.003$, large effect) (Fig. 2a), but were completely dissociated in the depressed subjects ($r(10) = 0.01$, $p = 0.98$, no effect) (Fig. 2b). Given that adipose tissue can be a source of circulating cytokines (Antuna-Puente et al., 2008), and that BMI scores were higher in depressed subjects, we wanted to investigate the possibility that the different inter-cytokine correlations observed in depressed participants and controls were secondary to BMI effects. Follow-up partial correlation analyses controlling for BMI effects did not change the results, as IL-10 and IL-6 remained uncorrelated in depressed participants ($r(9) = 0.16$, $p = 0.63$) and highly correlated in controls ($r(8) = 0.82$, $p = 0.004$).

4. Discussion

While it has been previously shown that major depression is associated with an increase in pro-inflammatory cytokines, the

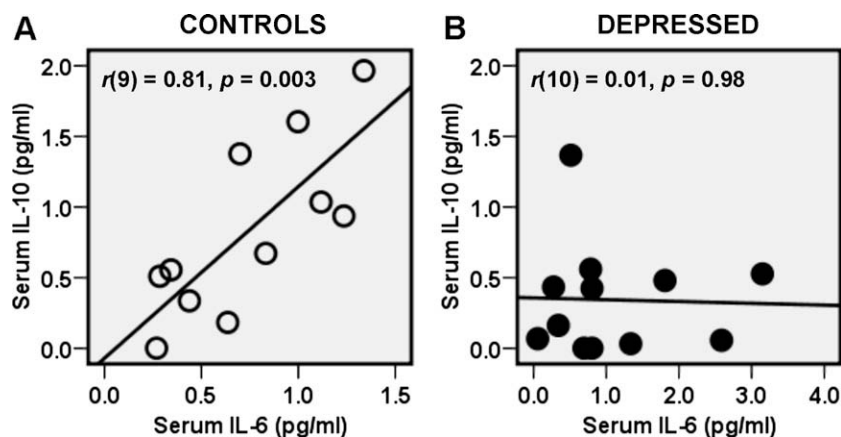


Fig. 2. Scatterplots of serum IL-6 and serum IL-10 concentrations in control (open circles, Panel A) versus depressed (closed circles, Panel B) subjects. To examine a potential deficiency in the feed forward loop between IL-6 and IL-10 in depressed subjects, we examined the strength and nature (positive, negative, or flat) of the correlations between circulating IL-6 and IL-10 concentrations in depressed subjects and controls. Serum IL-6 and IL-10 concentrations were strongly and positively correlated in controls subjects ($r(9) = 0.81$, $p = 0.003$, large effect. Panel A), but were completely dissociated in the depressed subjects ($r(10) = 0.01$, $p = 0.98$, no effect. Panel B).

role of anti-inflammatory cytokines has not been thoroughly investigated. Moreover, to our knowledge, the regulatory association between pro- and anti-inflammatory cytokines (represented here by the correlation between circulating IL-6 and IL-10 levels) in the context of depression has never before been examined. Given the potential importance of cytokines in mediating aspects of depression and its related co-morbid inflammatory diseases, the purpose of this study was to test the following hypotheses: first, we hypothesized that relative to controls, depressed subjects would exhibit lower circulating IL-10 concentrations, higher IL-6 concentrations and higher IL-6/IL-10 ratios. We also hypothesized that across the whole sample of participants, higher levels of depressive symptoms would similarly be associated with lower IL-10 and higher IL-6 concentrations, as well as higher IL-6/IL-10 ratios. Finally, we hypothesized that circulating IL-6 and IL-10 concentrations would be strongly and positively correlated in controls, whereas the correlation would be weaker or absent in depressed subjects. The results presented here generally confirm these hypotheses and lay the framework for their further testing, validation, and mechanistic evaluation.

Our results show that compared to controls, depressed subjects expressed significantly lower serum IL-10 concentrations, non-significantly higher IL-6 concentrations, and significantly higher IL-6/IL-10 ratios. Further, higher levels of depressive symptoms were significantly related to lower IL-10 concentrations and tended to be related to higher IL-6/IL-10 ratios, but were not significantly related to IL-6 concentrations, across the whole sample of participants. To our knowledge, this paper is the first to present the finding that control subjects showed a strong positive correlation between serum IL-6 and IL-10 concentrations, which was completely absent (near-zero effect size) in depressed subjects. Although depressed subjects had significantly higher BMI than the controls, co-varying for BMI (or controlling for BMI) did not alter this finding.

These results suggest that major depression is accompanied by a shift in overall cytokine balance towards pro-inflammatory cytokines, and by a disruption in the feed-forward relationship between IL-6 and IL-10. Under healthy conditions, this feed-forward loop is likely to result in an IL-6 driven induction of IL-10 release, which would in turn have the potential to dampen or resolve inflammatory processes through its immuno-regulatory/anti-inflammatory effects. Therefore, one important characteristic of depression, that needs to be further verified, may be the absence of a counter-balancing, immunoregulatory relationship between pro- and anti-inflammatory cytokines, which may partially mediate and/or contribute to the chronic inflammatory milieu that accompanies depression and its co-morbid disorders. It is possible that reduced induction and/or action of IL-10, may add to the depressogenic as well as the inflammatory disease-facilitating effects of chronic, low-level elevations in pro-inflammatory cytokines.

These results also suggest that circulating concentrations of IL-10, or the ratio of serum IL-6/IL-10 may be sensitive biomarkers of immune dysfunction in depression. However, the potential for IL-10 and IL-6/IL-10 ratio serving as biomarkers for depression needs to be further tested and validated. It must also be acknowledged that the low IL-10 concentrations in depressed subjects observed in this study contrast with other studies that found no group differences (Huang and Lee, 2007; O'Brien et al., 2007), or found increases in serum IL-10 concentrations in depressed adults (Simon et al., 2008). We currently do not have an explanation for the few inter-study differences that are known. However, findings similar to ours have been reported by a study showing that patients with major depression express significantly higher circulating concentrations of pro-inflammatory cytokines and significantly lower concentrations of anti-inflammatory cytokines and that these dif-

ferences are reversed following sertraline treatment (Sutcliffe et al., 2007). Furthermore, findings from numerous studies showing decreased mitogen-stimulated IL-10 production by blood leukocytes from depressed subjects (Rothermundt et al., 2001; Trzonkowski et al., 2004), and studies showing that anti-depressant treatment increases IL-10 production by *ex vivo* stimulated blood leukocytes (Kubera et al., 2001; Maes et al., 1999), also lend support to the results described here. Though not statistically significant, the modest elevations in IL-6 concentrations among the depressed subjects in our study are consistent with other case-controlled studies finding higher IL-6 concentrations in depressed adults (Maes et al., 1997; Miller et al., 2002; O'Brien et al., 2007; Pace et al., 2006) and studies showing a failure to suppress circulating pro-inflammatory cytokine levels in selective serotonin reuptake inhibitor (SSRI) resistant depressed patients (O'Brien et al., 2007). In addition, the small to medium-range effect size suggests that statistically significant differences in serum IL-6 concentrations between depressed and control subjects may have been found with a larger sample size.

It is important to recognize that lack of complete agreement between findings from different studies may be explained by the fact that depression is a heterogeneous disorder, and different subtypes of depression may have different psychological and physiological profiles. For example, different subtypes of depression may present with different profiles of HPA axis function and appetite (Andreasson et al., 2007), and studies suggest that melancholic and atypical depression may have different immunological profiles (Kaestner et al., 2005; Rothermundt et al., 2001).

Interestingly, an *in vitro* study examining lipopolysaccharide (LPS)-induced cytokine production by alveolar macrophages provides important clues about the potential mechanisms mediating the results described here. This study showed that *in vitro* exposure of an alveolar macrophage cell line to serotonin prior to stimulation with LPS resulted in a significant decrease in LPS-induced production of pro-inflammatory cytokines, and a significant increase in production of IL-10 (Menard et al., 2007). Similarly, Kubera et al. have shown a significant decrease in the IFN- γ /IL-10 ratio in supernatant obtained from human blood leukocytes that were treated with serotonin prior to stimulation with LPS (Kubera et al., 2000). Taken together, the studies described above lend support to the idea that the serotonin-deficient conditions that accompany major depression may contribute to the increased ratio of pro- to anti-inflammatory cytokines reported here. However, the complexity of the system needs to be further investigated because studies have also shown that pro-inflammatory cytokines and their actions can in turn precipitate serotonin deficiency by increasing the activity of indoleamine 2,3 dioxygenase (IDO) which diverts tryptophan metabolism towards the kynurenine pathway and decreases tryptophan availability for serotonin synthesis (Dantzer et al., 2008b; Miller, 2009; Miller et al., 2009; Schiepers et al., 2005).

Thus, the question of what came first in major depression, serotonin deficiency, or pro-inflammatory bias, still needs to be addressed. As with most complex biological systems, it is likely that the relationship is bi-directional: in some cases, the initiation of depressive symptoms leading to major depression may be mediated by an increase in pro-inflammatory cytokines and/or a decrease in anti-inflammatory cytokines, resulting in an overall increase in pro-inflammatory drive that results in serotonin depletion through activation of the IDO pathway described above (Dantzer et al., 2008b; Miller, 2009; Miller et al., 2009; Schiepers et al., 2005). This initiation pathway may lead to new-onset depression after the occurrence of inflammatory, autoimmune, and cardiovascular diseases. In other cases, the initiation of depressive symptoms may be mediated by a decrease in serotonin levels that then result in an increase in pro-inflammatory cytokine drive

through pathways similar to those described above (Kubera et al., 2000; Menard et al., 2007). Regardless of the initiating event, the reciprocal and feed-forward nature of the relationship between increased pro-inflammatory drive and serotonin depletion, could perpetuate a vicious cycle. Understanding the mechanistic components of such a feed-forward cycle is critical for designing effective therapeutics.

The importance of IL-10 is highlighted by critical actions that it is known to have, all of which would counter the depressogenic effects of increased pro-inflammatory cytokines and decreased serotonin: first, anti-inflammatory cytokines like IL-10 are critical for inhibiting potentially immuno-pathological actions of pro-inflammatory cytokines (Couper et al., 2008). Therefore, it is conceivable that IL-10 could also counter central nervous system related pro-inflammatory cytokine driven immuno-pathology that may contribute to depression. Second, during the later stages of an inflammatory response, IL-10 is important for negative feedback that reduces the expression of pro-inflammatory cytokines (Couper et al., 2008; Heyen et al., 2000). Therefore, IL-10 could potentially decrease the production of pro-inflammatory cytokines in illnesses like depression that are thought to involve chronic low-level inflammation. Third, IL-10 has been shown to suppress pro-inflammatory cytokine induced expression of IDO (Tu et al., 2005). Such IDO-inhibiting actions of IL-10 could shift tryptophan metabolism towards the serotonin synthesis pathway and ameliorate or restore serotonin deficiency. Taken together, these results suggest that IL-10, its synthetic analogs, or factors that result in endogenous increases in IL-10 are all potential therapeutic agents for depression. Support for this also comes from rodent studies showing that central administration of IL-10 significantly reduced LPS-induced sickness behaviors (Bluthe et al., 1999), that IL-10 over-expressing mice showed reduced, while IL-10 deficient mice showed increased, anxiety- and depressive-like behavior (Mesquita et al., 2008), and that IL-10 deficient mice showed exacerbated fatigue and motor deficits following LPS administration (Krzyszton et al., 2008). Therefore, the role of IL-10 and related agents that increase IL-10 levels or its actions, needs to be further investigated in larger studies with the goal of designing agents that maximize IL-10 induced inhibition of immuopathology, while minimizing or eliminating suppression of protective immune function.

Limitations of this study include the small sample size and cross sectional design. However, the fact that we detected significant group differences in serum IL-10 concentrations with a relatively under-powered analysis mitigates the potential risk of making a Type I error. Another limitation is the lack of measurement of other pro- and anti-inflammatory cytokines as well as Type-1 and Type-2 cytokines that may have provided a more comprehensive measure of disruption of cytokine-mediated immuno-regulatory feed-forward and feed-back mechanisms. Third, we do not present treatment data here. It would be of clear significance and would support our mechanistic interpretations if antidepressant treatment restores the IL-6 to IL-10 ratio as well as the normal counter-regulatory pattern of their release, and we are currently investigating this possibility. Finally, future higher-powered studies will be needed to fully investigate the potentially important contribution of BMI, that may be a mediator or a moderator of the findings reported here because the depressed group had higher BMI, and BMI serves as a proxy for greater intra-abdominal fat, an important source of inflammatory cytokines. In this regard, it is also important for future studies to examine the hypothesis that the association between pro-inflammatory cytokines and depression will be more strongly influenced by BMI than the association between anti-inflammatory cytokines and depression.

Clearly, further research is required for elucidating mechanisms and evaluating the clinical applicability of these findings. However, the findings presented here are important because they demon-

strate a significant decrease in IL-10 concentrations and an increased serum IL-6/IL-10 ratio in major depression. To our knowledge, this study is also the first to show that an important biological characteristic of major depression, may be the virtual absence of the robust and positive regulatory correlation between circulating IL-6 and IL-10 that is observed in control subjects. As such, these two parameters, IL-6/IL-10 ratio and IL-6 to IL-10 correlation, may serve as important biomarkers for major depression, and merit further investigation in larger studies. The usefulness of these immunological indices as potential biomarkers also comes from the fact that the cytokine measurements presented here were made directly on conventionally-obtained serum that is significantly more straightforward to collect and analyze than cytokine production by mitogen-stimulated immune cells. These results also suggest, that in addition to targeting reduction of pro-inflammatory cytokines therapeutically as a treatment for depression (Dantzer et al., 2008b; Raison et al., 2006), it may be useful therapeutically to induce controlled increases in anti-inflammatory cytokines like IL-10, to mimic conditions that effectuate their salubrious suppression of pro-inflammatory immunopathology without harmful suppression of protective immunity.

In summary, these data suggest that major depression is associated with a net pro-inflammatory state that may be related to a deficiency of anti-inflammatory/immuno-regulatory cytokines in addition to an excess of pro-inflammatory cytokines. Determining the mechanisms responsible for the apparent loss of counter-regulatory cytokine control may lead to new insights into the underlying pathophysiology of major depression and of its associated medical morbidities. Given the potential importance of these findings, future studies are needed to replicate these results, and to explore the potential for using a two-pronged intervention involving neurotransmitter-directed and immunomodulatory approaches for the treatment of depression.

Conflict of interest

The authors report no conflicts of interest.

Author contributions

Firdaus S. Dhabhar, conceptualized and designed immunological aspects of study, formulated hypotheses and analytical strategies, and was the lead writer of the manuscript. Heather M. Burke, designed and conceptualized the study with the PI, co-managed project, clinically assessed participants, led the data analysis, and co-wrote the manuscript. Elissa S. Epel, designed and conceptualized the study with the PI, guided data analysis, and contributed to writing the manuscript. Synthia H. Mellon, designed and conceptualized the study with the PI, managed biological specimen collection, handling and quality control, and contributed to writing the manuscript. Rebecca Rosser, co-managed project and reviewed drafts of the manuscript. Victor I. Reus, designed and conceptualized the study with the PI, clinically assessed and treated participants, and reviewed drafts of the manuscript. Owen M. Wolkowitz, was Principal Investigator, conceptualized and designed the study, was clinically responsible for participants, clinically assessed and treated participants, managed the project, analyzed data, guided data analysis, and co-wrote the manuscript. All authors contributed to, and have approved the final manuscript.

Grant support

This publication was supported by The O'Shaughnessy Foundation Grant (PI: OMW), a UCSF Academic Senate Grant (PI: OMW), and the NIH/NCRR UCSF-Clinical and Translational Science Insti-

tute (Grant Number UL1 RR024131), and by laboratory startup resources provided by the Carl and Elizabeth Naumann Fund (FSD). The funding agencies had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the funders.

Acknowledgements

We thank Jean M. Tillie for running ELISAs and generating cytokine data, Dr. Eve Kupferman for her help with diagnostic interviews and clinical evaluations, and Dr. J. Craig Nelson and Dr. Steven Hamilton for conducting psychiatric and medical evaluations and for assisting in the clinical care of subjects. We also thank the collegial members of the UCSF Depression Center, who helped with grant reviews, fund raising and subject referrals. We would like to acknowledge the outstanding nursing and laboratory support provided to this study by the UCSF Clinical and Translational Science Institute.

References

- Andreasson A, Arborelius L, Erlanson-Albertsson C, Lekander M. A putative role for cytokines in the impaired appetite in depression. *Brain, Behavior, and Immunity* 2007;21:147–52.
- Antuna-Puente B, Feve B, Fellahi S, Bastard JP. Adipokines: the missing link between insulin resistance and obesity. *Diabetes & Metabolism* 2008;34:2–11.
- Basterzi AD, Aydemir C, Kisa C, Aksaray S, Tuzer V, Yazici K, Goka E. IL-6 levels decrease with SSRI treatment in patients with major depression. *Human Psychopharmacology* 2005;20:473–6.
- Bluth RM, Castanon N, Pousset F, Bristow A, Ball C, Lestage J, Michaud B, Kelley KW, Dantzer R. Central injection of IL-10 antagonizes the behavioural effects of lipopolysaccharide in rats. *Psychoneuroendocrinology* 1999;24:301–11.
- Bruce TO. Comorbid depression in rheumatoid arthritis: pathophysiology and clinical implications. *Current Psychiatry Reports* 2008;10:258–64.
- Bull SJ, Huezio-Diaz P, Binder EB, Cubells JF, Ranjith G, Maddock C, Miyazaki C, Alexander N, Hotopf M, Cleare AJ, Norris S, Cassidy E, Aitchison KJ, Miller AH, Pariante CM. Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. *Molecular Psychiatry* 2008.
- Capuron L, Dantzer R. Cytokines and depression: the need for a new paradigm. *Brain, Behavior, and Immunity* 2003;17(Suppl. 1):S119–24.
- Capuron L, Miller AH. Cytokines and psychopathology: lessons from interferon-alpha. *Biological Psychiatry* 2004;56:819–24.
- Castanon N, Leonard BE, Neveu PJ, Yirmiya R. Effects of antidepressants on cytokine production and actions. *Brain, Behavior, and Immunity* 2002;16:569–74.
- Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *Journal of Immunology* 2008;180:5771–7.
- Daftarian PM, Kumar A, Kryworuchko M, Diaz-Mitoma F. IL-10 production is enhanced in human T cells by IL-12 and IL-6 and in monocytes by tumor necrosis factor-alpha. *Journal of Immunology* 1996;157:12–20.
- Dantzer R, Capuron L, Irwin MR, Miller AH, Ollat H, Perry VH, Rousey S, Yirmiya R. Identification and treatment of symptoms associated with inflammation in medically ill patients. *Psychoneuroendocrinology* 2008a;33:18–29.
- Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Reviews Neuroscience* 2008b;9:46–56.
- Evans DL, Charney DS, Lewis L, Golden RN, Gorman JM, Krishnan KR, Nemeroff CB, Bremner JD, Carney RM, Coyne JC, Delong MR, Frasure-Smith N, Glassman AH, Gold PW, Grant I, Gwyther L, Ironson G, Johnson RL, Kanner AM, Katon WJ, Kaufmann PG, Keefe FJ, Ketter J, Laughren TP, Leserman J, Lyketsos CG, McDonald WM, McEwen BS, Miller AH, Musselman D, O'Connor C, Petitto JM, Pollock BG, Robinson RG, Roose SP, Rowland J, Sheline Y, Sheps DS, Simon G, Spiegel D, Stunkard A, Sunderland T, Tibbitts Jr P, Valvo WJ. Mood disorders in the medically ill: scientific review and recommendations. *Biological Psychiatry* 2005;58:175–89.
- Fang Y, Sharp GC, Braley-Mullen H. Interleukin-10 promotes resolution of granulomatous experimental autoimmune thyroiditis. *American Journal of Pathology* 2008;172:1591–602.
- First MB, Spitzer RL, Gibbon M, Williams JBW. Structured clinical interview for DSM-IV-TR axis I disorders, research version, non-patient edition. New York: New York State Psychiatric Institute; 2002.
- Frasure-Smith N, Lesperance F, Irwin MR, Sauve C, Lesperance J, Theroux P. Depression, C-reactive protein and two-year major adverse cardiac events in men after acute coronary syndromes. *Biological Psychiatry* 2007;62:302–8.
- Glaser R, Robles TF, Sheridan J, Malarkey WB, Kiecolt-Glaser JK. Mild depressive symptoms are associated with amplified and prolonged inflammatory responses after influenza virus vaccination in older adults. *Archives of General Psychiatry* 2003;60:1009–14.
- Goodwin JS, Zhang DD, Ostir GV. Effect of depression on diagnosis, treatment, and survival of older women with breast cancer. *J Am Geriatr Soc* 2004;52:106–11.
- Hamilton M. A rating scale for depression. *Journal of Neurology Neurosurgery and Psychiatry* 1960;23:56–62.
- Heyen JR, Ye S, Finck BN, Johnson RW. Interleukin (IL)-10 inhibits IL-6 production in microglia by preventing activation of NF-kappaB. *Brain Research. Molecular Brain Research* 2000;77:138–47.
- Hjerl K, Andersen EW, Keiding N, Mouridsen HT, Mortensen PB, Jorgensen T. Depression as a prognostic factor for breast cancer mortality. *Psychosomatics* 2003;44:24–30.
- Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosomatic Medicine* 2009;71:171–86.
- Huang TL, Lee CT. T-helper 1/T-helper 2 cytokine imbalance and clinical phenotypes of acute-phase major depression. *Psychiatry and Clinical Neurosciences* 2007;61:415–20.
- Irwin MR, Miller AH. Depressive disorders and immunity: 20 years of progress and discovery. *Brain, Behavior, and Immunity* 2007;21:374–83.
- Kaestner F, Hettich M, Peters M, Sibrowski W, Hetzel G, Ponath G, Arolt V, Cassens U, Rothermundt M. Different activation patterns of proinflammatory cytokines in melancholic and non-melancholic major depression are associated with HPA axis activity. *Journal of Affective Disorders* 2005;87:305–11.
- Kenis G, Maes M. Effects of antidepressants on the production of cytokines. *International Journal of Neuropsychopharmacology* 2002;5:401–12.
- Kiecolt-Glaser JK, Glaser R. Depression and immune function: central pathways to morbidity and mortality. *Journal of Psychosomatic Research* 2002;53:873–6.
- Krzyszton CP, Sparkman NL, Grant RW, Buchanan JB, Broussard SR, Woods J, Johnson RW. Exacerbated fatigue and motor deficits in interleukin-10-deficient mice after peripheral immune stimulation. *American Journal of Physiology: Regulatory Integrative Comparative Physiology* 2008;295:R1109–14.
- Kubera M, Kenis G, Bosmans E, Scharpe S, Maes M. Effects of serotonin and serotonergic agonists and antagonists on the production of interferon-gamma and interleukin-10. *Neuropsychopharmacology* 2000;23:89–98.
- Kubera M, Lin AH, Kenis G, Bosmans E, van Bockstaele D, Maes M. Anti-inflammatory effects of antidepressants through suppression of the interferon-gamma/interleukin-10 production ratio. *Journal of Clinical Psychopharmacology* 2001;21:199–206.
- Larson SJ, Dunn AJ. Behavioral effects of cytokines. *Brain, Behavior, and Immunity* 2001;15:371–87.
- Maes M. Evidence for an immune response in major depression: a review and hypothesis. *Progress in neuro-psychopharmacology & biological psychiatry* 1995;19:11–38.
- Maes M, Bosmans E, De Jongh R, Kenis G, Vandoolaeghe E, Neels H. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine* 1997;9:853–8.
- Maes M, Song C, Lin AH, Bonaccorso S, Kenis G, De Jongh R, Bosmans E, Scharpe S. Negative immunoregulatory effects of antidepressants: inhibition of interferon-gamma and stimulation of interleukin-10 secretion. *Neuropsychopharmacology* 1999;20:370–9.
- Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *Public Library of Science (PLoS): Medicine* 2006;3:e442.
- McCaffery JM, Frasure-Smith N, Dube M-P, Theroux P, Rouleau GA, Duan Q, Lesperance F. Common genetic vulnerability to depressive symptoms and coronary artery disease: a review and development of candidate genes related to inflammation and serotonin. *Psychosomatic Medicine* 2006;68:187–200.
- Menard G, Turmel V, Bissonnette EY. Serotonin modulates the cytokine network in the lung: involvement of prostaglandin E2. *Clinical and Experimental Immunology* 2007;150:340–8.
- Mesquita AR, Correia-Neves M, Roque S, Castro AG, Vieira P, Pedrosa J, Palha JA, Sousa N. IL-10 modulates depressive-like behavior. *Journal of Psychiatric Research* 2008;43:89–97.
- Miller AH. Norman cousins lecture. Mechanisms of cytokine-induced behavioral changes: psychoneuroimmunology at the translational interface. *Brain, Behavior, and Immunity* 2009;23:149–58.
- Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biological Psychiatry* 2009.
- Miller GE, Stetler CA, Carney RM, Freedland KE, Banks WA. Clinical depression and inflammatory risk markers for coronary heart disease. *American Journal of Cardiology* 2002;90:1279–83.
- Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, Greiner K, Nemeroff CB, Miller AH. Paroxetine for the prevention of depression induced by high-dose interferon alpha. *New England Journal of Medicine* 2001;344:961–6.
- Musselman DL, Nemeroff CB. Depression really does hurt your heart: stress, depression, and cardiovascular disease. *Progress in Brain Research* 2000;122:43–59.
- O'Brien SM, Scully P, Fitzgerald P, Scott LV, Dinan TG. Plasma cytokine profiles in depressed patients who fail to respond to selective serotonin reuptake inhibitor therapy. *Journal of Psychiatric Research* 2007;41:326–31.
- Ogawa Y, Duru EA, Ameredes BT. Role of IL-10 in the resolution of airway inflammation. *Current Molecular Medicine* 2008;8:437–45.
- Onitilo AA, Nietert PJ, Egede LE. Effect of depression on all-cause mortality in adults with cancer and differential effects by cancer site. *General Hospital Psychiatry* 2006;28:396–402.

- Onyike CU, Crum RM, Lee HB, Lyketsos CG, Eaton WW. Is obesity associated with major depression? results from the third national health and nutrition examination survey. *American Journal of Epidemiology* 2003;158:1139–47.
- Pace TW, Mletzko TC, Alagbe O, Musselman DL, Nemeroff CB, Miller AH, Heim CM. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *American Journal of Psychiatry* 2006;163:1630–3.
- Penninx BW, Kritchewsky SB, Yaffe K, Newman AB, Simonsick EM, Rubin S, Ferrucci L, Harris T, Pahor M. Inflammatory markers and depressed mood in older persons: results from the health, aging and body composition study. *Biological Psychiatry* 2003;54:566–72.
- Pollak Y, Yirmiya R. Cytokine-induced changes in mood and behaviour: implications for 'depression due to a general medical condition', immunotherapy and antidepressive treatment. *International Journal of Neuropsychopharmacology* 2002;5:389–99.
- Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends in Immunology* 2006;27:24–31.
- Rothermundt M, Arolt V, Fenker J, Gutbrodt H, Peters M, Kirchner H. Different immune patterns in melancholic and non-melancholic major depression. *European Archives of Psychiatry and Clinical Neuroscience* 2001;251:90–7.
- Rush AJ, Giles DE, Schlesser MA, Fulton CL, Weissenburger J, Burns C. The inventory for depressive symptomatology (IDS): preliminary findings. *Psychiatry Research* 1986;18:65–87.
- Schatzberg AF. Recent studies of the biology and treatment of depression. *Focus* 2005;3:14–24.
- Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. *Progress in Neuropsychopharmacology & Biological Psychiatry* 2005;29:201–17.
- Simon NM, McNamara K, Chow CW, Maser RS, Papakostas GI, Pollack MH, Nierenberg AA, Fava M, Wong KK. A detailed examination of cytokine abnormalities in Major Depressive Disorder. *European Neuropsychopharmacology* 2008;18:230–3.
- Sjogren E, Leanderson P, Kristenson M, Ernerudh J. Interleukin-6 levels in relation to psychosocial factors: studies on serum, saliva, and in vitro production by blood mononuclear cells. *Brain, Behavior, and Immunity* 2006;20:270–8.
- Stommel M, Given BA, Given CW. Depression and functional status as predictors of death among cancer patients. *Cancer* 2002;94:2719–27.
- Suarez EC. Joint effect of hostility and severity of depressive symptoms on plasma interleukin-6 concentration. *Psychosomatic Medicine* 2003;65:523–7.
- Sutcgil L, Oktenli C, Musabak U, Bozkurt A, Cansever A, Uzun O, Sanisoglu SY, Yesilova Z, Ozmenler N, Ozsahin A, Sengul A. Pro- and anti-inflammatory cytokine balance in major depression: effect of sertraline therapy. *Clinical and Developmental Immunology* 2007;2007:76396.
- Triantafyllou N, Evangelopoulos ME, Kimiskidis VK, Kararizou E, Boufidou F, Fountoulakis KN, Siamouli M, Nikolaou C, Sfagos C, Vlaikidis N, Vassilopoulos D. Increased plasma homocysteine levels in patients with multiple sclerosis and depression. *Annals of General Psychiatry* 2008;7:17.
- Trzonkowski P, Mysliwska J, Godlewska B, Szmít E, Lukaszuk K, Wieckiewicz J, Brydak L, Machala M, Landowski J, Mysliwski A. Immune consequences of the spontaneous pro-inflammatory status in depressed elderly patients. *Brain, Behavior, and Immunity* 2004;18:135–48.
- Tu H, Rady PL, Juelich T, Smith EM, Tyring SK, Hughes TK. Cytokine regulation of tryptophan metabolism in the hypothalamic-pituitary-adrenal (HPA) axis: implications for protective and toxic consequences in neuroendocrine regulation. *Cellular & Molecular Neurobiology* 2005;25:673–80.
- 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.
- Watkins LR, Maier SF. Immune regulation of central nervous system functions: from sickness responses to pathological pain. *Journal of Internal Medicine* 2005;257:139–55.
- Whooley MA, Caska CM, Hendrickson BE, Rourke MA, Ho J, Ali S. Depression and inflammation in patients with coronary heart disease: findings from the heart and soul study. *Biological Psychiatry* 2007;62:314–20.
- Wichers MC, Kenis G, Koek GH, Robaey G, Nicolson NA, Maes M. Interferon- α -induced depressive symptoms are related to changes in the cytokine network but not to cortisol. *Journal of Psychosomatic Research* 2007;62:207–14.
- Wulsin LR, Vaillant GE, Wells VE. A systematic review of the mortality of depression. *Psychosomatic Medicine* 1999;61:6–17.
- Zautra AJ, Yocum DC, Villanueva I, Smith B, Davis MC, Attrep J, Irwin M. Immune activation and depression in women with rheumatoid arthritis. *Journal of Rheumatology* 2004;31:457–63.