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

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

\textsuperscript{a}Department of Psychiatry and Behavioral Sciences, Stanford University, School of Medicine, USA
\textsuperscript{b}Stanford Institute for Immunity, Transplantation and Infection, Stanford University School of Medicine, USA
\textsuperscript{c}Health Psychology Program, Department of Psychiatry, University of California, San Francisco, School of Medicine, USA
\textsuperscript{d}Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, School of Medicine, USA
\textsuperscript{e}Department of Psychiatry, Langley Porter Psychiatric Institute, University of California, San Francisco, School of Medicine, USA

\textsuperscript{*}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).

\textsuperscript{1}FSD and HMB contributed equally to this work.

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

\textsuperscript{p}\textsuperscript{2}0022-3956/$ - see front matter \textsuperscript{c}\textsuperscript{3}2009 Elsevier Ltd. All rights reserved.

do:10.1016/j.jpsychires.2009.05.010

\textsuperscript{2}E-mail address: dhabhar@gmail.com (F.S. Dhabhar).

\textsuperscript{3}FSD and HMB contributed equally to this work.
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 $\geq 17$ (HAM-D; (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 $>99^\circ F$) or elevated WBC counts (WBC $>10,000$), (4) immunizations within 4 weeks of the blood draw, (5) psychotropic medication use (including antidepressants, mood stabilizers, 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 ($\pm 2$–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...
controls (Mann–Whitney $U = 31.00, z = -2.16, p = .03, \text{Cohen's } d = 0.86$). 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 $20.58 \pm 3.09$ (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 conducted. After a complete description of the study to the initial contact with subjects, the study was explained, and a

### 2.3. Cytokine assays

Samples were collected in 10 ml SST tubes (Becton Dickinson, Franklin Lakes, NJ). Serum was frozen and stored at $-80^\circ$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 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 ($z = 0.05$) 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 $< d < 0.50; 0.10 < r < 0.25$), “medium effect” (0.50 $< d < 0.80; 0.25 < r < 0.40$), and “large effect” ($d > 0.80; r > .40$).

### 3. Results

Across the entire sample of age-matched subjects, there were no significant correlations between age and serum concentrations of IL-10 ($r(21) = -0.34, p = .11$) or IL-6 ($r(21) = -0.29, p = .19$). 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 ($mean = 0.34 \pm 0.11, median = 0.29$ pg/ml) compared to controls ($mean = 0.83 \pm 0.19, median = 0.67$ 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 ($mean = 1.09 \pm 0.28, median = 0.79$ pg/ml) compared to controls ($mean = 0.74 \pm 0.12, median = 0.70$ 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-

### Table 1

Sample characteristics by group.

<table>
<thead>
<tr>
<th>Means (SD)</th>
<th>Controls ($n = 11$)</th>
<th>Depressed ($n = 12$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.00 (13.27)</td>
<td>38.42 (11.03)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)$^*$</td>
<td>24.37 (3.61)</td>
<td>28.67 (5.95)</td>
</tr>
<tr>
<td>Inventory of Depressive Symptoms$^*$</td>
<td>5.64 (5.28)</td>
<td>33.58 (10.07)</td>
</tr>
<tr>
<td>Hamilton Depression Rating Scale</td>
<td>20.58 (3.09)</td>
<td></td>
</tr>
</tbody>
</table>
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
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 differences are reversed following sertaline treatment (Sutclig 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 (Andreasen 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 deple
tion 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 inhibition of potentially immuno-pathological actions of pro-inflammatory cytokines, could shift tryptophan metabolism in 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 inhibition of potentially immuno-pathological actions of pro-inflammatory cytokines, could shift tryptophan metabolism in 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 inhibition of potentially immuno-pathological actions of pro-inflammatory cytokines, could shift tryptophan metabolism in 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 inhibition of potentially immuno-pathological actions of pro-inflammatory cytokines, could shift tryptophan metabolism in 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 inhibition of potentially immuno-pathological actions of pro-inflammatory cytokines, could shift tryptophan metabolism in inflammation.

Support for this also comes from rodent studies showing that central administration of IL-10 significantly decreased 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 immuno-pathology, 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 feedback 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 demonstrate 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. Eipel, designed and conceptualized the study with the PI, co-managed project, clinically assessed and treated participants, managed the project, 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. Tilly 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


