Dissociation of inflammatory markers and natural killer cell activity in major depressive disorder

Jennifer L. Pike, Michael R. Irwin

Cousins Center for Psychoneuroimmunology, University of California, Los Angeles, Neuropsychiatric Institute, Los Angeles, CA 90095-7057, USA

Received 17 April 2005; received in revised form 22 May 2005; accepted 22 May 2005

Available online 14 July 2005

Abstract

Major depressive disorder is associated with increases in infectious disease risk as well as the incidence of inflammatory disorders. Declines of natural killer (NK) cell activity are reliably found in depression, whereas other studies report evidence of inflammation in depressed patients. The potential association between NK activity and circulating markers of immune activation has not been previously examined in the context of major depression. In this study, we measured levels of NK activity, circulating levels of interleukin-6 (IL-6), soluble interleukin-2 receptor, and acute phase proteins in 25 male patients with current major depressive disorder and 25 age, gender, and body weight comparable controls. As compared to controls, patients with major depressive disorder showed lower NK activity (p < .05) and higher circulating levels of IL-6 (p < .05). Levels of NK activity were not correlated with IL-6 or with other markers of immune activation. The independent effect of depression on inflammatory markers and natural killer immune responses has implications for understanding individual differences in the adverse health effects of major depressive disorder.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Major depressive disorder; Immunity; Natural killer cell activity; Inflammation

1. Introduction

Major depressive disorder, which exceeds a lifetime incidence of 10% (Kessler et al., 2003; Michaud et al., 2001), is a potent risk factor for disease morbidity with depressed persons showing a mortality rate twice that found in nondepressed persons (Penninx et al., 1999; Rudolph and Nemeroff, 2003; Wulsin et al., 1999). Altered functioning of the immune system is implicated as a mechanism that might contribute to medical morbidity of major depressive disorder including risk of infectious disease (Evans et al., 2002) as well as inflammatory disorders (Zautra et al., 2004). For example, depressed persons show reductions of cellular and innate immune responses that are associated with infectious disease susceptibility (Cohen and Miller, 2000; Leserman, 2003), whereas other studies have found that depression is linked to immune activation in patients with inflammatory disorders such as rheumatoid arthritis (Zautra et al., 2004) and cardiovascular disease (Lesperance et al., 2004; Miller et al., 2002a,b). Indeed, several recent meta-analyses have found that major depression is reliably associated with a reduction of natural killer (NK) cell activity and with increases of circulating levels of the proinflammatory cytokine, interleukin-6 (IL-6), and possibly other markers of immune activation such as acute proteins (e.g., haptoglobin) (Herbert and Cohen, 1993; Irwin, 2002; Zorrilla et al., 2001). However, despite the number of studies that have evaluated different aspects of immunity in depression, no study has simultaneously examined the association between NK activity and inflammatory markers in the context of major depression (Raison and Miller, 2003); rather, these immune differences have been generated in separate samples of depressed patients. In this

* Corresponding author. Fax: +1 310 794 9247.

E-mail address: mirwin1@ucla.edu (M.R. Irwin).
study, we evaluate levels of NK activity as well as markers of immune activation including circulating levels of IL-6, soluble interleukin-2 receptor (sIL-2R), and acute phase proteins (haptoglobin, α-1-antitrypsin, and α-1-acid glycoprotein) in acutely depressed patients as compared to age-, gender, and body weight matched comparison controls.

2. Materials and methods

2.1. Human subjects

Depressed subjects were recruited by flyers and self-referral, fulfilled Diagnostic, and Statistical Manual-IV (DSM-IV) criteria for Major Depressive Disorder, current episode (American Psychiatric Association, 2000), and underwent immunological assessment prior to inclusion in treatment trials. Control subjects were recruited by advertisements, fulfilled DSM-IV criteria for Never Mentally Ill (American Psychiatric Association, 2000), and matched to depressed subjects on the basis of age (± 5 years), gender, and body weight. The total sample was comprised of 25 control subjects and 25 depressed patients; all subjects were male.

Participants were in good health as determined by medical history and laboratory screening blood tests. None fulfilled criteria for current alcohol or substance abuse or dependence, although 10 depressed subjects had lifetime histories of alcohol abuse/dependence in full remission for greater than 6 months. No other major psychiatric co-morbidity was identified in the depressed group. None of the subjects reported recent (<10 days) viral illness, or used immunosuppressive medications such as corticosteroids. Four depressed subjects reported prior treatment with antidepressant and/or anxiolytic medications and reported use of these medication 19–60 days prior to evaluation; the other 21 depressed subjects did not report such psychotropic medication use in the last 6 months. Other medication use was infrequent: one depressed patient and one control reported the daily use of a diuretic medication for hypertension, and one other depressed patient reported use of a β-agonist inhalant for asthma symptoms 7 days prior to evaluation.

2.2. Procedures

Psychiatric diagnoses of depressed and control subjects were made following administration of the Schedule for Clinical Interview and Diagnosis (SCID)—DSM-III or DSM-IV and consensus meeting of psychiatrists, clinical psychologists, research fellows, and nursing staff. Tobacco histories were obtained using a previously validated interview for substance dependence histories (Bucholz et al., 1994). Severity of depressive symptoms was measured using the Hamilton Depression Rating Scale (Williams, 2001).

Blood sampling for assay of NK activity and plasma levels of immune activation was obtained in the morning between 6 a.m. and 9 a.m. After placement of a 21 gauge intravenous catheter, subjects rested in a recliner chair for 30 min and blood samples were obtained.

2.3. Immune assays

As previously described, peripheral blood mononuclear cells were isolated and NK cell activity was assayed using a standard chromium51 labeled K562 cytotoxicity assay, with results expressed as percentage specific cytotoxicity across four effector to target (E:T) cell ratios (40:1; 20:1; 10:1; and 5:1) (Jung and Irwin, 1999). Values of NK activity from nine pairs of controls and depressed patients have been previously reported (Irwin et al., 2003), although these previous results were obtained on a different assay day. Serum levels of IL-6 were quantified by means of enzyme-linked immunosorbent assay methods (R&D Systems, Minneapolis, MN) with all samples from matched pairs assayed at the same time, in a single run with a single lot number of reagents and consumables employed by a single operator, with intra-assay coefficients of variation for all variables less than 5%. Serum levels of sIL-2R were measured using Quantikine Immunoassay kits (R&D Systems, Minneapolis, MN). Nephelometry was used to measure serum levels of the acute phase proteins, haptoglobin, α-1-antitrypsin (AAT), and α-1-acid glycoprotein (AAG) (Dade Behring, Marburg, Germany). For the full set of immune activation markers, values of IL-6, IL-2R, haptoglobin, AAT, and AAG have not been previously reported.

2.4. Statistical analyses

Paired t tests were used to assess differences in age, body weight, severity of depressive symptoms, and serum levels of IL-6, sIL-2R, haptoglobin, AAT, and AAG between the depressed and control subjects. Values of IL-6 and AAG were square root transformed to achieve normality. A 2 (group: controls, depressed subjects) × 4 (E:T ratios) repeated measures ANOVA tested group differences in NK activity. Pearson correlations evaluated associations between the immune measures.

3. Results

3.1. Subject characteristics

The controls and depressed subjects were similar in age (42.7 ± 12.0 vs. 42.5 ± 9.2 years; t(24) = 0.4, p = 97) and in body weight (181.9 ± 20.0 vs. 175.6 ± 24.8 lbs;
As compared to controls, depressed subjects reported significantly more depressive symptoms as measured by Hamilton total scores (0.6 ± 1.0 vs. 24.5 ± 6.5; \( t(24) = 16.9 \), \( p < .001 \)). None of the controls were current tobacco smokers, whereas the depressed group had 14 smokers and 11 nonsmokers. Hamilton scores were similar in the depressed smokers and depressed nonsmokers. Alcohol consumption histories in the last 3 months were similar in controls vs. depressed subjects as indexed by average number of drinks per day (1.7 ± 1.3 vs. 1.4 ± 1.7; \( t(24) = 0.6 \), \( p = .52 \)), number of drinking days per month (14.4 ± 15.2 vs. 9.5 ± 13.5; \( t(24) = 1.2 \), \( p = .24 \)), and days since last drink (13.5 ± 25.6 vs. 30.8 ± 35.1; \( t(24) = 1.9 \), \( p = .06 \)).

### 3.2. NK activity and inflammatory markers in major depression

Table 1 shows group differences in NK activity and in serum levels of IL-6, sIL-2R, and the acute phase proteins, haptoglobin, ATT, and AAG. As compared to controls, NK activity was significantly lower in the depressed group (\( p = .05 \)). In contrast, circulating levels of IL-6 were elevated in the depressives vs. controls (\( p < .05 \)). Serum levels of sIL-2R, haptoglobin, AAT, and AAG did not differ between the two groups.

### 3.3. Smoking status and history of alcohol dependence in major depression

To evaluate the effects of current tobacco smoking on NK activity and IL-6, the depressed group was stratified into current smokers (\( n = 14 \)) vs. nonsmokers (\( n = 11 \)). In addition, to examine the effects of alcohol abuse/dependence histories on these immune variables, separate analyses were done in which the depressed group was stratified by the presence (\( n = 10 \)) or absence of co-morbid alcohol abuse/dependence history (\( n = 15 \)). Neither levels of NK activity nor IL-6 differed between the depressed smokers vs. depressed nonsmokers or between the depressed patients with and without a history of alcohol abuse/dependence (\( p’s > .1 \)).

### 3.4. Dissociation between NK activity and immune activation

Correlations were used to test the association between NK activity and serum levels of immune activation markers. Within the total sample, NK activity (20:1 E:T ratio) was not correlated with IL-6 (\( r = -0.03 \), \( p = .84 \)), sIL-2R (\( r = -0.17 \), \( p = .26 \)), haptoglobin (\( r = 0.25 \), \( p = .10 \)), AAT (\( r = -0.17 \), \( p = .29 \)) or AAG (\( r = 0.18 \), \( p = .24 \)). Similar nonsignificant correlations were found between NK activity at other E:T ratios and markers of immune activation. Within the depressed group, NK activity (20:1 E:T ratio) was not correlated with IL-6 (\( r = 0.13 \), \( p = .59 \)), sIL-2R (\( r = -0.12 \), \( p = .58 \)), haptoglobin (\( r = 0.29 \), \( p = .16 \)), AAT (\( r = 0.42 \), \( p = .06 \)) or AAG (\( r = -0.20 \), \( p = .19 \)). Within the control group, we also found no correlations between NK activity (20:1 E:T ratio) and IL-6 (\( r = -0.46 \), \( p = .84 \)), sIL-2R (\( r = -0.17 \), \( p = .44 \)), haptoglobin (\( r = 0.20 \), \( p = .36 \)), AAT (\( r = 0.03 \), \( p = .91 \)) or AAG (\( r = -0.03 \), \( p = .09 \)).

Additional analyses were conducted to explore whether there was a nonlinear association between NK activity and markers of immune activation. The sample was stratified using a median split of NK activity (20:1 E:T ratio); the two groups with high or low NK activity had similar levels of IL-6 (\( t(48) = 0.40 \), \( p = .69 \)), sIL-2R (\( t(48) = 1.1 \), \( p = .27 \)), haptoglobin (\( t(48) = 1.4 \), \( p = .17 \)), AAT (\( t(48) = 0.76 \), \( p = .45 \)), and AAG (\( t(48) = 1.1 \), \( p = .26 \)). Likewise, when the sample was stratified using a median split of IL-6, the two groups with high or low IL-6 levels had similar levels of NK activity (\( F(1,48) = 0.47 \), \( p = .50 \)).

Levels of IL-6 were correlated with AAG (\( r = 0.33 \), \( p < .05 \)) and haptoglobin was correlated with AAT (\( r = 0.39 \), \( p < .01 \)) and with AAG levels (\( r = 0.49 \), \( p < .001 \)).

### 3.5. Adjustment for smoking status and body weight in the correlations

To determine whether the dissociation between NK activity and markers of immune activation was influenced by smoking status, a series of partial correlations were performed controlling for current smoking status; partial correlations controlling for smoking status found no statistically significant correlations between NK activity and IL-6, sIL-2R, haptoglobin, AAT, or AAG in the total sample or within the depressed group (all

### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (( n = 25 ))</th>
<th>Major depressive disorder (( n = 25 ))</th>
<th>( F )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Group effect</td>
</tr>
<tr>
<td>NK activity (% cytotoxicity)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E:T 40:1</td>
<td>39.3</td>
<td>16.5</td>
<td>30.0</td>
<td>17.3</td>
</tr>
<tr>
<td>E:T 20:1</td>
<td>26.0</td>
<td>12.0</td>
<td>19.6</td>
<td>12.1</td>
</tr>
<tr>
<td>E:T 10:1</td>
<td>16.1</td>
<td>7.5</td>
<td>11.7</td>
<td>7.1</td>
</tr>
<tr>
<td>E:T 5:1</td>
<td>9.0</td>
<td>4.5</td>
<td>6.3</td>
<td>4.4</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sIL-2R (pg/ml)</td>
<td>852.9</td>
<td>317.9</td>
<td>1105.3</td>
<td>1070.9</td>
</tr>
<tr>
<td>Haptoglobin (mg/dl)</td>
<td>92.3</td>
<td>47.9</td>
<td>97.1</td>
<td>50.7</td>
</tr>
<tr>
<td>AAT (mg/dl)</td>
<td>105.4</td>
<td>21.2</td>
<td>123.2</td>
<td>45.9</td>
</tr>
<tr>
<td>AAG (mg/dl)</td>
<td>74.7</td>
<td>24.0</td>
<td>78.7</td>
<td>23.5</td>
</tr>
</tbody>
</table>

\( a \) E:T refer to Effector to Target cell ratio.

\( b \) Analyses on square-root transformed values.
Furthermore, it is possible that the contribution of adipose tissue to levels of IL-6 may differ across the groups and confound the correlation between NK activity and markers of immune activation, even though the groups were not different in body weight. Thus, partial correlations controlling for body weight were used to test for the relationship between NK activity and IL-6, sIL-2R, haptoglobin, AAT, and AAG; no statistically significant correlations were found for these measures in the total sample or within the depressed or control groups (all \( p's > .10 \)).

4. Discussion

This is the first study to examine the association between levels of NK activity and plasma markers of immune activation in the context of major depression. Similar to meta-analytic findings (Zorrilla et al., 2001), patients with major depression show declines of NK activity and increases of plasma levels of IL-6 as compared to controls. Levels of NK activity were not correlated with IL-6 or with other markers of immune activation. In contrast, IL-6 is known to induce acute phase proteins, and IL-6 levels correlated with AAG. Finally, no depression differences were found for acute phase protein levels or sIL-2r, consistent with meta-analytic reports that alterations of these immune parameters are preliminary and/or not consistent between laboratories (Zorrilla et al., 2001).

It is not known what mechanisms might account for increases of IL-6 levels in some depressed patients and for decreases of NK activity in other patients with depression. Raison and Miller (2003) have proposed that depressed patients show inadequate glucocorticoid-mediated feedback inhibition of immune activation; for example, increased concentrations of plasma IL-6 positively correlate with post-dexamethasone suppression test (DST) cortisol levels in depression (Lowy et al., 1988). In contrast, several studies of patients with major depression have failed to show a relationship between altered NK activity and plasma or urinary concentrations of free cortisol (Irwin et al., 1988; Kronfol et al., 1986; Miller et al., 1991). Taken together, these data suggest that glucocorticoid resistance in depression is associated with immune activation (Miller et al., 2002a,b; Raison and Miller, 2003), yet not with declines of NK activity. Hence, it is speculated that insufficiency in glucocorticoid signaling of inflammation might identify a group of depressed patients at risk for exacerbations of inflammatory disorders such as rheumatoid arthritis (Raison and Miller, 2003).

Genetic and metabolic variation in the expression of proinflammatory cytokines (Kiecolt-Glaser et al., 2003) as well as decreases of NK activity (Pike et al., 1997), stress-induced increases of plasma C-reactive protein is reported to occur only in stressed persons who have the A allele of tumor necrosis factor \( \alpha \)-308 G/A polymorphism (Jeanmonod et al., 2004). Likewise, polymorphism of the 174 bp upstream of the transcription initiation site of the IL6 gene, the 174G/C allele, correlates with plasma IL6 levels (Fishman et al., 1998), although the relationship of this polymorphism with major depression or with NK immune responses is not known. Finally, one-third of total IL-6 in the circulation is estimated to originate from adipose tissue (Mohamed-Ali et al., 1998). Even in depressed patients and controls who are similar in body weight and/or adiposity, metabolic alterations in adipose tissue signaling might contribute to increases of IL-6 in depression independent of immune cell production of this proinflammatory cytokine.

Our study has several limitations. First, the control group was self-selected in response to recruitment drives and may not be representative of the general population. Second, consistent with the methods of prior studies that have described immune alterations in depressed patients, the control subjects had no lifetime history of a major psychiatric disorder. Thus, the immunological differences between the controls and depressed patients might be viewed as related to the absence of psychiatric morbidity in the controls rather than positively associated with depression. Third, the study sample included only males. Findings of reduced NK activity in depressed persons may be gender specific as suggested by Evans et al. (1992), in which decreases of NK activity and NK cell numbers are found in male but not female depressed subjects as compared to gender matched controls. Fourth, no inference can be made about whether the decline of NK activity is due to decreases in the function of NK cells or a decline in their number, although with the recent accumulation of studies of depressed patients, robust declines in the number of circulating NK cells, as well as T and B cells, have not been reported in depression (Zorrilla et al., 2001). Fifth, the prevalence rates of smoking and of lifetime incidence of alcohol dependence are elevated in depressed patients as compared to normal individuals (Irwin et al., 1990; Jung and Irwin, 1999), and only the depressed group had representation of smokers and those co-morbid for alcoholism histories. No differences in NK activity or IL-6 levels were found within the depressives stratified either by smoking status or by co-morbidity for alcohol dependence histories. Although it appears that smoking status is not related to further increases of IL-6 in depression (Lesperance et al., 2004; Motivála et al., 2005), we have previously reported greater declines of NK activity in depressed smokers as compared to depressed nonsmokers, and lower NK activity in depressed patients co-morbid for alcohol dependence histories as compared
to depressives without such alcohol dependence histories. However, given prior effects sizes (Irwin et al., 1990; Jung and Irwin, 1999), a sample size of at least 70 patients is needed for sufficient statistical power to identify potential additive effects for smoking status and/or alcohol dependence history in depression.

This study provides evidence for inflammation in major depression, which is not correlated with declines of NK cell responses in these patients. The independent effect of depression on inflammatory markers as compared to NK responses implies for understanding individual differences in the adverse health effects of major depressive disorder.

Acknowledgments

This work was supported in part by grants AA13239, DA16541, MH55253, AG18367, T32-MH19925, M01-RR00865, M01 RR00827, General Clinical Research Centers Program, and the Cousins Center for Psychoneuroimmunology.

References


