

## Differences in cytokines between non-suicidal patients and suicidal patients in major depression

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### Abstract

Several studies have shown that there is an imbalance between pro-inflammatory and anti-inflammatory cytokines in major depressive disorder (MDD). However, little is known about the role of cytokines in suicide. In the present study, amounts of IL-6, IL-2, IFN- $\gamma$ , IL-4, and TGF- $\beta$ 1 produced by mitogen-stimulated whole blood were measured in 36 MDD patients who had recently attempted suicide, 33 non-suicidal MDD patients, and 40 normal controls. The severity of depression symptoms and suicidal behaviors was evaluated using Hamilton's depression rating scale (HDRS), the Lethality Suicide Attempt Rating Scale (LSARS), and the Risk-Rescue Rating (RRR). Non-suicidal MDD patients had significantly higher IL-6 production than suicidal MDD patients and normal controls ( $p < 0.001$ ). Suicidal MDD patients had significantly lower IL-2 compared with non-suicidal patients and normal controls ( $p < 0.001$ ). Both MDD groups, with or without attempted suicide, had significantly lower IFN- $\gamma$  and IL-4 and higher TGF- $\beta$ 1 production. HDRS scores had significant positive correlations with IL-6, IFN- $\gamma$ , and the Th1/Th2 ratio and significant negative correlations with IL-4 in non-suicidal depression patients ( $p < 0.005$ ); however, these correlations did not hold true for suicidal patients. Suicidal MDD patients had no significant correlations between the LSARS or RRR scores and cytokine release. Our findings suggest that the immune response has distinct differences between non-suicidal patients and suicidal patients. Non-suicidal MDD may be associated with increased IL-6 production and a Th1/Th2 imbalance with a shift to Th1, while suicidal MDD may be associated with decreased IL-2.

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**Keywords:** Cytokine; Depression; IL-2; IL-4; Suicide

### 1. Introduction

Suicide is a significant public health issue and a major cause of death throughout the world. The WHO estimates that suicide

accounts for almost 2% of deaths worldwide (WHO, 2000). Over 90% of suicide victims and suicide attempters have a psychiatric illness (Robins et al., 1959). About 60% of all suicides occur in to the context of mood disorders (Robins et al., 1959). Even in the psychiatric groups at the highest risk, most patients never attempt suicide, indicating the importance of a diathesis or predisposition to suicidal behavior that is independent of the main psychiatric disorder (Mann, 2003).

Our current knowledge about the neurobiology of suicide is still limited. To date, many studies have suggested that at least 3 neurobiological systems are involved in the pathogenesis of suicidal behavior: a deficiency of the serotonergic system (Mann

*Abbreviations:* BDI, Beck Depression Inventory; BMI, body mass index; HDRS, the Hamilton's depression rating scale; IFN, interferon; IL, Interleukin; LSARS, the Lethality Suicide Attempt Rating Scale; MDD, major depressive disorder; RRR, the Risk-Rescue Rating; STAI, State-Trait Anxiety Inventory; TGF, Transforming growth factor; Th1, the T helper type 1 cell; Th2, the T helper type 2 cell; Th3, the T helper type 3 cell.

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et al., 1996), a hyperactivity of the hypothalamic–pituitary–adrenal axis (Yerevanian et al., 2004), and an excess release of norepinephrine followed by a deficiency of norepinephrine (Ordway et al., 1994). In addition, several studies have shown the involvement of neurotrophic factors (Karege et al., 2005) and cholesterol (Kim and Myint, 2004). Some findings about suicidal behavior have shown a close similarity to the pathogenesis of major depression. Others have shown distinctive neurobiological findings in suicidal behavior. For example, disturbed serotonergic functioning is known to be associated with major depression and suicides. However, several studies have suggested that major depression is associated with serotonergic disturbances in several cortical and subcortical areas, whereas the abnormality in suicidal depression is localized to parts of the prefrontal cortex (Mann, 2003).

Several immune changes in major depression have been reported, including activation of the inflammatory response system, increased concentrations of pro-inflammatory cytokines, and increases in prostaglandin E2 and negative immunoregulatory cytokines in peripheral blood (Kim et al., 2002; Leonard, 2001; Maes, 1999). Several studies have shown that there is an imbalance between pro-inflammatory and anti-inflammatory cytokines in major depression (Anisman and Merali, 2003; Kim and Maes, 2003). Some have reported increased release of pro-inflammatory cytokines, interleukin (IL)-1, IL-2, and IL-6 by activated macrophages and interferon (IFN)- $\gamma$  by activated T cells (Ackerman et al., 1998; Maes, 1995, 1995, 1999). A depressive syndrome that may include suicidal attempt is a common complication of the IFN- $\alpha$  therapy in hepatitis C (Dieperink et al., 2004; Kraus et al., 2003). Depression related to cytokine administration is attenuated by antidepressant treatments.

Our previous study, evaluating the plasma IFN- $\gamma$ , IL-4, and TGF- $\beta$ 1 of depressed patients and normal controls, showed a higher IFN- $\gamma$ /IL-4 ratio in depressed patients before treatment (Myint et al., 2005). We wonder whether suicidal depressed patients might have a similar profile of cytokines to that in non-suicidal depressed patients, or whether distinctive features might be associated with the cytokine profile of suicidal patients. In the present study, we examined IL-6 (a pro-inflammatory cytokine), IL-2, IFN- $\gamma$  (T helper type 1 cell (Th1) cytokines), IL-4 (a Th2 cytokine), and transforming growth factor- $\beta$ 1 (a TGF- $\beta$ 1) (Th3 cytokine) produced by mitogen-stimulated whole blood in major depression patients who had recently attempted suicide, non-suicidal depressive patients, and normal controls. We compared the immune response in non-suicidal and suicidal depression groups with controls and examined the correlations between the cytokines and the severity of depression or lethality of the suicide attempt.

## 2. Methods

### 2.1. Subjects

Seventy subjects were patients admitted to emergency rooms of 3 university hospitals (Korea University Ansan Hospital, Soonchunhyang University Seoul Hospital, and Soonchun-

hyang University Chun-An Hospital) following suicide attempts between August 2003 and February 2006. Initial psychiatric interviews were conducted within 24 h after admission. We defined attempted suicide as self-harm behaviors with at least some intent to end one's life. Subjects were excluded if their self-injurious behaviors were determined to have no suicidal intention or ideation. We excluded subjects with psychiatric comorbidities of Axis I or Axis II, such as bipolar disorder, schizophrenia, alcohol abuse, substance abuse, or personality disorders. We also excluded subjects with general medical problems, such as a common cold, influenza, or any other inflammation. Individuals were evaluated independently by trained psychiatrists using a Structured Clinical Interview for DSM-IV (First et al., 1998) and Hamilton's 17-item depression rating scale (HDRS) (Hamilton, 1960) for the purpose of establishing a DSM-IV criteria diagnosis (APA, 1994). All patients had a HDRS total score of 15 or higher. Diagnoses and ratings were decided by mutual consent (blind to cytokine concentrations). The evaluations consisted of reviews of psychiatric and medical histories, including current and previous medications and alcohol intake. Among 70 suicidal patients, we finally selected 36 patients (10 males and 26 females; mean age =  $34.0 \pm 9.2$  years) who were either medication-naive or medication-free for at least 8 weeks, were diagnosed with unipolar major depressive disorder (MDD) according to the DSM-IV criteria, and had HDRS scores over 15.

Two control groups were established for this study. The first group included hospitalized non-suicidal depressive patients who were either medication-naive or medication-free for at least 8 weeks. The 33 patients (13 males and 20 females; mean age =  $35.7 \pm 12.0$  years) selected for this study were closely matched with individual patients in the suicidal group in terms of age and sex. They met all the DSM-IV criteria for unipolar major depression and had HDRS scores of over 15. They all had no history of attempted suicide within one year when included in this study.

The 40 normal controls (13 males and 25 females; mean age =  $34.5 \pm 10.2$  years) consisted of randomly selected healthy individuals who visited the Korea University Ansan Hospital for regular health screenings. They were carefully matched with the other 2 groups in terms of age and sex. Healthy volunteers had no self-reported personal or familial psychiatric history or medication history. The volunteers were required to have scores below 10 on the Beck Depression Inventory (BDI) and below 40 on the State-Trait Anxiety Inventory (STAI) in order to be included in this study.

No statistically significant differences were noted across the 3 groups for age, sex, or body mass index (BMI). The demographic characteristics of the 3 groups are summarized in Table 1. The study protocol was approved by the Ethics Committee of Korea University, and written informed consent was obtained from each individual.

### 2.2. Clinical evaluation

A trained psychologist (HJW) evaluated the lethality of each individual suicide attempt using Weisman and Worden's risk-

rescue rating (RRR) system (Weisman and Worden, 1972) and the Lethality Suicide Attempt Rating Scale-updated (LSARS-II) (Berman et al., 2003; Smith et al., 1984). The RRR system (Weisman and Worden, 1972) is a descriptive and quantitative method of assessing the lethality of suicidal acts. According to this system, lethality can be expressed as the ratio of 5 risk and 5 rescue factors. Each factor is rated on a scale of 1 to 3 points and the total points of the risk factors or the rescue factors are then converted to a risk score or a rescue score, respectively. The RRR score is determined by the formula  $[A/(A+B)] \times 100$ , in which  $A$  is the risk score and  $B$  is the rescue score.

The LSARS (Berman et al., 2003; Smith et al., 1984) is an 11-point scale (0 = “death is an impossible result” to 10 = “death is almost certain”). Each point on the scale has comprehensive descriptive anchors that incorporate both the lethality of the means and the context or circumstances of the event. In our suicidal patients, the means and standard deviation of the RRR and LSARS scores were  $30.79 \pm 13.62$  and  $3.55 \pm 1.89$ , respectively.

In this study, 22 (61.1%) of patients had attempted suicide by pharmacological methods, namely drug ingestion ( $n=15$ , 41.7%) and pesticide or agricultural chemical ingestion ( $n=7$ , 19.5%), and 14 (28.9%) had used non-pharmacological methods, namely wrist cutting with superficial laceration ( $n=3$ , 8.3%), wrist cutting with deep laceration ( $n=8$ , 22.2%), and hanging ( $n=3$ , 8.3%). There were no significant differences between those using pharmacological methods vs. those using non-pharmacological methods in subtype of depression ( $\chi^2=0.835$ ,  $p=0.361$ ) or severity of depression (HDRS total score) ( $t=-0.331$ ,  $p=0.743$ ). Among the 36 suicide attempters, 21 patients (58.3%) had attempted suicide for the first time, 9 (83.3%) had made 1 previous attempt, 5 (13.9%) had made 2 previous attempts, and 1 (2.8%) had made 5 previous attempts.

The time since the attempt, which is defined as the time interval from attempt to arrival at a hospital, was divided into ‘less than 1 h’ ( $n=5$ ), ‘1–4 h’ ( $n=18$ ), and ‘more than 4 h’ ( $n=13$ ).

The severity of depressive symptoms was also evaluated using Hamilton’s 17-item depression rating scale (HDRS) (Table 1).

### 2.3. Blood sample collection

Following an overnight fast, blood samples were drawn from the subjects’ antecubital veins between 0800 and 0900 h for psychiatric controls without medication and normal controls. For the suicidal patients, blood was collected at the time of admission to the emergency room after the failed attempt. Approximately 20 ml of blood was collected and placed in a lithium heparin vacuum tube.

For the suicidal patients, we recorded the time of day when each subject’s blood was taken in the following time blocks: early morning (0800–0959;  $n=6$ ), late morning (1000–1359;  $n=4$ ), afternoon (1400–1859;  $n=2$ ), evening (1900–2359;  $n=7$ ), first half of the night (2400–0359,  $n=8$ ), and second half of the night (0400–0759;  $n=9$ ). The cytokine levels of the suicidal patients were compared among time blocks, and only plasma IL-6 levels were significantly higher in the morning than in other time blocks ( $\chi^2=18.039$ ,  $p=0.003$ ).

#### 2.3.1. Cytokine assays

Human cytokines IL-6, IL-2, IL-4, IFN- $\gamma$ , and TGF- $\beta$ 1 were examined by stimulating whole blood with phytohemagglutinin (PHA) and lipopolysaccharide (LPS) in culture supernatant (De Groote et al., 1992). A mixture of 750  $\mu$ l of RPMI-1640 medium with l-glutamine (Biowhittaker, Walkersville, Maryland, USA) supplemented with 10% fetal bovine serum (FBS, Gibco BRL, USA, Invitrogen, Carlsbad, CA, USA) and 100 IU/ml penicillin (Sigma, Saint Louis, MO, USA), 100  $\mu$ g/ml streptomycin (Sigma), and 250  $\mu$ l of whole blood were placed into 24-well cell culture plates. PHA (4  $\mu$ g/ml; Sigma) and LPS (20  $\mu$ g/ml; Sigma) were added. Samples were incubated for 48 h in a humidified atmosphere at 37 °C, 5% CO<sub>2</sub>. After incubation, supernatants were taken off carefully under sterile conditions, divided into eppendorf tubes, and frozen immediately at –70 °C until thawed for assay. Human cytokines IL-6, IL-2, IL-4, IFN- $\gamma$ , and TGF- $\beta$ 1 were assayed using the DuoSet ELISA Development System (R&D Systems, Minneapolis, MN, USA). All assays were carried out by the same operator using the recommended buffers, diluents, and substrates. The concentrations of the samples in each plate were calculated according to each standard curve and the dilution factor. The

Table 1  
The demographic characteristics of normal controls, non-suicidal depressive patients, and suicidal depressive patients

Variables	Normal controls (N=40)	Non-Suicidal depression (N=33)	Suicidal depression (N=36)	<i>p</i>
Age (years)	34.5±12.2	35.7±12.0	34.0±9.2	0.776 <sup>a</sup>
Sex (Male/Female)	15/25	13/20	10/26	0.544 <sup>a</sup>
BMI	21.61±2.78	22.07±4.14	21.13±2.63	0.070 <sup>a</sup>
Age at onset (years)		36.8±11.3	33.3±9.7	0.232 <sup>b</sup>
HDRS total score		27.7±6.2	22.2±4.7	0.001 <sup>b</sup>
Subtype of depression (N)				
Melancholic type		29	28	0.348 <sup>b</sup>
Atypical type		4	8	

Data are presented as mean±SD.

HDRS, Hamilton’s 17-item depression rating scale.

The mean difference is significant at the 0.05 level.

<sup>a</sup> It is a comparison among 3 groups, which are normal controls, non-suicidal depression, and suicidal depression.

<sup>b</sup> It is a comparison between non-suicidal depression and suicidal depression.

intra- and inter-assay coefficients of variation for all analyses were less than 8%.

#### 2.4. Statistical analysis

The distribution of numerical variables was analyzed separately in each group to establish parametric vs. nonparametric requirements. Study groups were compared for continuous variables by a 2-tailed *t*-test and ANOVA. The Bonferroni method was used for *post hoc* comparisons. Also, *in vitro* IL-2 production and Th1/Th2 ratios were analyzed by using the Mann–Whitney *U* test and Kruskal–Wallis test and the Dunnett C method for *post hoc* comparisons. These statistics were used because IL-2 production and Th1/Th2 ratios, though continuous variables, were not normally distributed, and our sample size was not sufficiently large. For discrete variables, study groups were compared by a chi-square test. The General Linear Model command of the SPSS was used when controlling for covariates. Correlations among cytokine production, Th1/Th2 ratios, HDRS scores, LSARS, and RRR scores were calculated using Pearson's or Spearman's correlation coefficients. A 2-tailed probability of less than 0.05 was considered statistically significant ( $p < 0.05$ ). All statistical analyses were performed with SPSS version 12.0 for Windows.

### 3. Results

#### 3.1. *In vitro* IL-6, IFN- $\gamma$ , IL-2, IL-4, and TGF- $\beta$ 1 production in suicidal MDD patients, non-suicidal MDD patients, and normal controls

MDD patients with or without attempted suicide had significantly lower production of IFN- $\gamma$  and IL-4 compared with normal controls ( $F = 7.526$ ,  $df = 2$ , 106,  $p = 0.001$ ;  $F = 11.591$ ,  $df = 2$ , 106,  $p < 0.001$ , respectively). Non-suicidal MDD patients had significantly higher IL-6 production than suicidal patients and normal controls ( $F = 33.437$ ,  $df = 2$ , 106,  $p < 0.001$ ). The production of IL-2 was significantly lower in suicidal MDD patients than in non-suicidal MDD patients and normal controls ( $\chi^2 = 25.177$ ,  $p < 0.001$ ). However, the release of TGF- $\beta$ 1 was significantly higher in MDD patients both with

and without attempted suicide than in normal controls ( $F = 10.367$ ,  $df = 2$ , 106,  $p < 0.001$ ) (Table 2). For the Th1/Th2 ratio, suicidal MDD patients had a significantly lower IL-2/IL-4 ratio compared with non-suicidal MDD patients and normal controls ( $\chi^2 = 11.888$ ,  $p = 0.003$ ). Non-suicidal MDD patients had a higher IL-2/IL-4 ratio than normal controls, but the difference was not statistically significant. IFN- $\gamma$ /IL-4 ratios were higher in both depressed groups compared with normal controls, but the difference was not significant ( $\chi^2 = 0.734$ ,  $p = 0.693$ ) (Table 2).

Except for IL-6, the differences in cytokines and Th1/Th2 ratio between non-suicidal and suicidal patients were not attributable to the severity of depression (HDRS scores) or time of day when each subject's blood was taken, when controlling for covariates (data not shown). IL-6 was higher in non-suicidal depression than in suicidal depression ( $p = 0.011$ ) and IL-6 was higher in suicidal depression than in normal controls ( $p = 0.021$ ), when controlling for blood sampling time as a covariate. There were no significant differences in cytokine levels according to the subtype of major depression, the method of suicide attempt, or the time since the attempt in suicidal patients (data not shown).

#### 3.2. Correlations among cytokines, depression symptoms, and suicidal severity in MDD patients with or without attempted suicide

Among all 69 depressive patients, total HDRS scores had significantly positive correlations with IL-6 and IFN- $\gamma$  ( $r = 0.355$ ,  $p = 0.009$ ;  $r = 0.291$ ,  $p = 0.035$ , respectively), while they had a significantly negative correlation with IL-4 ( $r = -0.288$ ,  $p = 0.037$ ). Th1/Th2 ratios (IL-2/IL-4 or IFN- $\gamma$ /IL-4) were positively correlated with total HDRS scores ( $r_s = 0.347$ ,  $p = 0.012$ ;  $r_s = 0.418$ ,  $p = 0.002$ , respectively). Non-suicidal MDD patients had positive correlations between total HDRS scores and IFN- $\gamma$  ( $r = 0.444$ ,  $p = 0.026$ ), and negative correlations between total HDRS scores and IL-4 ( $r = -0.484$ ,  $p = 0.014$ ), and these differences were significant. Also, there were significantly positive correlations between total HDRS scores and Th1/Th2 ratios (IL-2/IL-4 or IFN- $\gamma$ /IL-4) in non-suicidal MDD patients ( $r_s = 0.423$ ,  $p = 0.044$ ;  $r_s = 0.691$ ,  $p < 0.001$ , respectively).

Table 2

The *in vitro* IL-2, IFN- $\gamma$ , IL-4, and TGF- $\beta$ 1 production among normal controls (NC), non-suicidal depressive patients (NS), and suicidal depressive patients (S)

(pg/mL)	Normal controls ( $n = 40$ )	Non-suicidal depression ( $n = 33$ )	Suicidal depression ( $n = 36$ )	$p$	<i>Post hoc</i> test
IL-6	234.19 $\pm$ 33.19	442.21 $\pm$ 167.66	278.26 $\pm$ 105.99	<0.001	NC, S < NS <sup>a,b</sup>
IL-2	242.77 $\pm$ 242.17	226.02 $\pm$ 274.63	76.61 $\pm$ 97.78	<0.001 <sup>c</sup>	NC, NS > S <sup>d</sup>
IFN- $\gamma$	506.69 $\pm$ 247.41	362.06 $\pm$ 288.03	293.87 $\pm$ 193.64	0.001	NC > NS, S <sup>a</sup>
IL-4	279.12 $\pm$ 100.23	183.98 $\pm$ 111.13	182.27 $\pm$ 89.59	<0.001	NC > NS, S <sup>a</sup>
TGF- $\beta$ 1	563.56 $\pm$ 283.54	944.12 $\pm$ 480.71	853.29 $\pm$ 357.71	<0.001	NC < NS, S <sup>a</sup>
IL-2/IL-4	1.00 $\pm$ 1.16	1.75 $\pm$ 2.49	0.78 $\pm$ 2.37	0.003 <sup>c</sup>	NL, NS > S <sup>d</sup>
IFN- $\gamma$ /IL-4	1.94 $\pm$ 1.02	4.94 $\pm$ 7.29	2.71 $\pm$ 5.62	0.693 <sup>c</sup>	

Data are presented as mean  $\pm$  SD.

<sup>a</sup> The Bonferroni method was used for *post hoc* comparisons.

<sup>b</sup> IL-6 was higher in non-suicidal depression than in suicidal depression ( $p = 0.011$ ) and IL-6 was higher in suicidal depression than in normal controls ( $p = 0.021$ ), when controlling blood sampling time as a covariate (NC < S < NS).

<sup>c</sup> Those data were analyzed by using the Kruskal–Wallis test.

<sup>d</sup> The Dunnett C method was used for *post hoc* comparisons.

However, suicidal MDD patients had no significant correlations between cytokines and HDRS scores. The LSARS and RRR scores were not significantly correlated with cytokines in suicidal MDD patients.

#### 4. Discussion

The major finding of this study is that MDD patients with recently attempted suicide had significantly lower *in vitro* production of IL-2, while non-suicidal patients had significantly higher release of IL-6. Compared with normal controls, both MDD groups had significantly decreased IFN- $\gamma$  and IL-4 and increased TGF- $\beta$ 1 production.

Our non-suicidal depressed patients had increased IL-6 and TGF- $\beta$ 1 production and decreased IFN- $\gamma$  and IL-4. Moreover, IL-6, IFN- $\gamma$ , and Th1/Th2 ratios had significant positive correlations with the severity of depression (HRDS total score), while IL-4 had a significant negative correlation. Some of our findings support the recent hypothesis that major depression may be related to increased IL-6 (Dunn et al 2005). IL-6 was also higher in suicidal depression patients than in normal controls ( $p=0.021$ ), when controlling sampling-time. Some studies have reported positive correlations between plasma levels of cytokines (IL-6 or IL-1 $\beta$ ) and depressive symptom severity (Alesci et al., 2005; Miller et al 2002; Thomas et al 2005). Both depressive symptoms and syndromal depression were reported to be associated with increased plasma IL-6 levels (Lutgendorf et al 1999). Moreover, a potential role for IL-6 was demonstrated by an animal study which found that a spontaneous increase in serum IL-6 concentrations in lupus-prone mice coincided with increased immobility in the forced swim test and that sucrose intake was also decreased when IL-6 was over-expressed systemically in healthy mice (Sakic et al 2001).

Several studies have reported increased cytokines, including IL-6, IL-1 $\beta$ , IL-2, and IFN- $\gamma$ , in severely depressed patients (Anisman et al 1999; Maes, 1999; Mullar and Ackenheil 1998; Sluzewska 1999), while a recent report showed significantly lower IL-2 and IFN- $\gamma$  (Th1) and higher IL-4 and IL-13 (Th2) in MDD patients compared with normal controls (Pavon et al 2006). The results of prior studies are not entirely consistent. Our results also showed decreased IFN- $\gamma$  and IL-4 production and no alteration in IL-2 in non-suicidal depressed patients. However, Th1/Th2 ratios had significant positive correlations with the severity of depression in our non-suicidal depressed patients. The positive correlations between Th1/Th2 and the severity of depression indicated an imbalance of Th1/Th2 cytokines or a Th1 shift in major depression (Anisman et al 2005; Calcagni and Elenkov 2006; Lee and Kim 2006; Myint et al., 2005).

Another finding in this study was that suicidal and non-suicidal MDD patients had different alterations in cytokines. IL-2 release and the IL-2/IL-4 ratio in suicidal patients were significantly lower than those in non-suicidal MDD patients and normal controls. Suicidal patients had no significant correlations between HDRS scores and any cytokine or the Th1/Th2 ratio. Thus, we speculate that suicidal depression, unlike non-

suicidal MDD, may not be associated with an increased Th1 cytokine and a Th1/Th2 imbalance with a shift to Th1. However, our results are not entirely consistent with previous findings. High-dose IL-2 administration was associated with suicide in one case report (Baron et al., 1993). Plasma soluble IL-2 receptor in medication-free suicide attempters across psychiatric diagnoses (mainly mood disorders) was reported to be at high levels in inpatients and remain high during follow-up as outpatients (Nassberger and Traskman-Bendz 1993). It is possible that IL-2 might constitute a neurobiological factor in suicide and that the suicidal attempt itself might cause the altered cytokine production; exploring these possibilities will require further study.

Our data showed increased *in vitro* TGF- $\beta$ 1 production in MDD patients both with and without attempted suicide. However, this was inconsistent with our previous findings, which indicated increased plasma TGF- $\beta$ 1 in depressed patients, not before treatment, but after treatment (Lee and Kim 2006; Myint et al., 2005). There were differences in the measuring methods or type of blood samples among these studies. There have been few studies about TGF- $\beta$ 1 in major depression. Stressors can activate the hypothalamic–pituitary–adrenal axis and the noradrenergic system and then increase the activity of glucocorticoids. Glucocorticoids enhance the synthesis of TGF- $\beta$  in human T cells (Batuman et al 1995). TGF- $\beta$  helps to orchestrate immune homeostasis at multiple levels and to maintain the balance of Th1 and Th2 cytokines (Aoki et al 2005; Zhang et al 2006).

Some studies have shown evidence of circadian or diurnal rhythms in cytokine levels in body fluids (Petrovsky and Harrison, 1998; Vgontzas et al., 2005). In healthy young adults, IL-6, IL-2, and IFN- $\gamma$  have a sleep-associated increase, and their peak production occurs during the night and early morning (Marshall and Born, 2002; Petrovsky and Harrison, 1998; Vgontzas et al., 2005). The circadian rhythm of cytokines might be affected by sleep, sleep deprivation, and stress. The circadian rhythm of IL-6 in major depression was reported to be shifted by 12 h (Alesci et al., 2005). In this study, however, blood was collected at different times in suicidal depressed and non-suicidal depressed patients, and this was one of our limitations. We sampled the blood of suicidal patients at the time of admission to the emergency room in order to exploit the immediate or acute response of cytokines to attempted suicide. There was significant diurnal variation of IL-6 in suicidal depression in our data, which is consistent with a previous report (Alesci et al., 2005), although the number of our subjects was too small to confirm that finding. Thus, we reanalyzed our data controlling for blood sampling time as a covariate.

The present study has other limitations. First, we did not thoroughly exclude the effects of various confounding factors from our data. The confounding factors include demographic characteristics such as smoking habits, medical status, diagnostic subtype, and the immunological parameters selected for each study. All these factors may contribute to the heterogeneous findings seen across many studies (Haack et al., 1999). We tried to control some confounding factors such as diagnostic subtype. However, smoking habits, psychosocial stresses, and the

immunological parameters were not controlled. Potential differences could exist between those who agreed to participate and those that did not, and this would be an important confounding factor. Another limitation is that we measured *in vitro* mitogen-stimulated cytokine production, not *in vivo* serum or plasma levels of cytokines, and the measurements were taken only one time before the treatment of MDD began. Some of our results were inconsistent with our previous findings (Lee and Kim 2006; Myint et al., 2005). If we had examined both the plasma concentrations and the mitogen-stimulated production of cytokines, we might have obtained more comprehensive data, including correlations between the *in vivo* and *in vitro* changes. Also, measuring the cytokines during the follow-up period might have allowed us to determine whether the changes in the cytokines reflect the state or the trait of MDD.

The present findings indicate that the immune profile in MDD has distinct differences between non-suicidal patients and suicidal patients. Major depression patients without a recent attempted suicide may have increased IL-6 production, while recent suicide attempts may be associated with decreased IL-2. Non-suicidal depression may be associated with a Th1/Th2 imbalance with a shift to Th1, while suicidal depression may not. Future studies should reveal the longitudinal course of these biological or immune factors in the context of major depression and suicide.

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