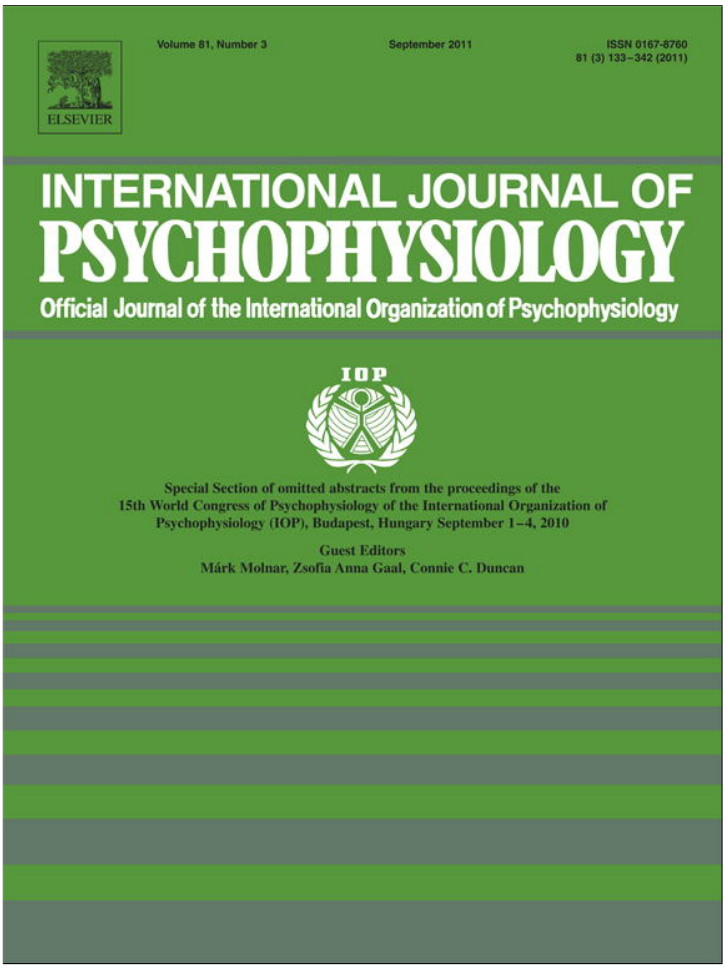

International Journal of Psychophysiology 81 (2011) 191–197

Contents lists available at ScienceDirect

International Journal of Psychophysiology

journal homepage: www.elsevier.com/locate/ijpsycho

Psychological distress, depressive symptoms, and cellular immunity among healthy individuals: A 1-year prospective study

Akinori Nakata [a,*], Masahiro Irie [b], Masaya Takahashi [c]

[a] National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Cincinnati, OH, USA
[b] Institute of Health Science, Kyushu University, Fukuoka, Japan
[c] National Institute of Occupational Safety and Health, Kawasaki, Japan

ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form 17 June 2011
Accepted 20 June 2011
Available online 6 July 2011

Keywords:
Psychological distress
Depressive symptoms
Immune system
Natural killer cells
Lymphocyte

ABSTRACT

Cross-sectional and case-control studies have reported that psychological distress and depression are associated with reduced cellular immune competence but the directionality of the relationship remains uncertain. This study investigated whether levels of psychological distress and depressive symptoms are related to subsequent changes in counts of lymphocyte subsets (natural killer (NK), B, and T cell) and/or whether changes of immune markers predict psychological distress/depressive symptoms in a 1-year prospective study design. A total of 105 healthy employees (67 men and 38 women), aged 23–59 (mean 40) years with an average of 15 years of education, underwent a blood draw for the measurement of circulating immune cells and completed the Japanese version of the 28-item General Health Questionnaire (GHQ-28) and the Center for Epidemiologic Studies Depression Scale (CES-D) in April 2002 (time 1) and 2003 (time 2). Hierarchical multiple linear regression analyses revealed that GHQ-28 and CES-D scores at time 1 were significantly ($p<.05$) and inversely associated with NK cells at time 2 controlling for potential confounders including time 1 NK cells ($\beta = -.221$ and $-.177$, respectively). In contrast, NK cells and NK cell cytotoxicity at time 1 did not predict GHQ-28 or CES-D score at time 2 controlling for GHQ-28/CES-D score at time 1. GHQ-28 and CES-D scores were not related to T or B cells at times 1 and 2. The present findings indicate that psychological distress and depressive symptoms may precede and predict suppression of NK cell immunity while NK cells did not lead to subsequent psychological distress and depressive symptoms, suggesting an absence of the bi-directional relationships.

Published by Elsevier B.V.

1. Introduction

According to the World Health Organization (WHO), depressive disorders were the third leading cause of burden of disease in 2004 but are projected to be placed first by the year 2030 (WHO, 2008). Depressive disorders are identified as an independent risk factor for all-cause mortality (Cuijpers and Smit, 2002) and major medical illnesses including cardiovascular diseases (CVD) (Nicholson et al., 2006) and cancer (Pinquart and Duberstein, 2010). Substantial evidence suggests that depressive disorders are likely to involve several pathophysiological pathways and considerable attention is now paid to the possible role of immunological dysregulation in the pathogenesis of depressive disorders (Irwin and Miller, 2007).

A number of cross-sectional/case-control studies have reported that psychological distress and depression are associated with reduced cellular immune competence (Bauer et al., 1995; Evans et al., 1992; Frank et al., 2002; Jung and Irwin, 1999; Park et al., 2006; Schleifer et al., 1989; Tsuboi et al., 2005). According to several meta-analytic reviews (Herbert and Cohen, 1993; Weisse, 1992; Zorrilla et al., 2001), depressive disorders are associated with decreased natural killer (NK) cells, impaired NK cell cytotoxicity (NKCC), poorer proliferative response of lymphocyte to mitogens, and elevated CD4/CD8 ratios. Although the conclusion of meta-analysis studies implies that depression suppresses cellular immune function, a large part of literatures included in the analyses were cross-sectional/case–control studies which preclude causal inference.

To date, there are only a limited amount of studies that investigated the association between psychological distress/depression and cellular immunity using longitudinal approach (Amati et al., 2010; Evans et al., 2002; Fortes et al., 2003; Irwin et al., 1992; Leserman et al., 1997; McGuire et al., 2002). For example, in an 18-month prospective study, community-dwelling older adults with chronic but mild depressive symptoms had lower response to T cell proliferation tests compared to non-depressed counterparts from baseline to follow-up (McGuire et al., 2002). Another study in an elderly population (aged 65+ years) found a decrease in the relative

* Corresponding author at: Division of Applied Research and Technology, MS-C24, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226, USA.
E-mail addresses: nakataa-tky@umin.ac.jp, cji5@cdc.gov (A. Nakata), irie@ihs.kyushu-u.ac.jp (M. Irie), takaham@h.jniosh.go.jp (M. Takahashi).

0167-8760/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ijpsycho.2011.06.009

proportion of activated CD4+ (CD4+DR+) and CD8+ (CD8+DR+) T cells but not NK, CD4+, or CD8+ T cells in depressed compared to non-depressed individuals over a period of four years (Fortes et al., 2003). A series of follow-up studies in human immunodeficiency virus-infected patients demonstrated that depressive symptoms were consistently associated with decline of NK cell immunity (Evans et al., 2002; Leserman et al., 1997). A study of healthy nurses revealed that those who exhibited increased psychological distress (as measured by a 12-item General Health Questionnaire (GHQ)) had decreased CD4+ T cells and increased CD8+ and CD8+CD57+ T cells compared to those who maintained lower psychological distress after 1-year follow-up (Amati et al., 2010).

As such, psychological distress/depressive symptoms seem to be associated with suppression of NK and T cell function over time, however, there is an important question that remained unanswered, that is, whether or not psychological distress/depressive symptoms and cellular immune function are bi-directionally related even after adjustment for baseline dependent variable and extensive potential confounders such as health behaviors, physical condition, work-related factors, and medications. This question seems important because it has been debated that depression and immunity may have bi-directional relationships (Bjerkeset et al., 2011; Gimeno et al., 2009; Howren et al., 2009; Matthews et al., 2010; Pike and Irwin, 2006; Steptoe et al., 2003; Stewart et al., 2009) and depression has been characterized as a disorder of both immune suppression (defined as reduced proliferative responses of immune cells and impaired innate and adaptive immunity) and immune activation (defined as the proliferation of immune cells and the increased production of proinflammatory cytokines) (Blume et al., 2011).

Considering the above findings, the current study was designed to investigate the association of psychological distress and depressive symptoms with cellular immune markers in a 1-year longitudinal study design. Our purpose was to clarify the following two specific questions: (1) do levels of psychological distress/depressive symptoms at baseline associated with subsequent cellular immune competency adjusted for baseline immune variables? (2) Do immunological variables at baseline related to subsequent psychological distress/depressive symptoms adjusted for baseline levels of psychological distress and depressive symptoms?

We measured circulating NK, B, and T cells as well as NKCC because these markers are known to reflect quantitative and qualitative aspects of cellular immunity, reported to be associated with psychosocial factors including depression, and are commonly used indicators in human psychoimmunologic studies (Herbert and Cohen, 1993; Segerstrom and Miller, 2004; Weisse, 1992; Zorrilla et al., 2001). With regard to functional roles of selected lymphocytes, T and B cells bear central roles in cellular and humoral immunity; subsets of T (CD4+ and CD8+) cells control production of immunoglobulins from B cells and secretion of cytokines. NK cells are large granular cells possessing killer activity against certain tumor cells and virus-infected cells without prior sensitization.

2. Methods

2.1. Study participants

The study was conducted as a part of annual occupational health examinations in April 2002 and 2003. All participants were full-time Japanese employees working in daytime with an average of 15 years of education. A total of 217 employees who underwent health examination were invited to participate in this study and the survey questionnaire including purpose, instruction, and informed consent was given to them in April, 2002 (Baseline, time 1). Overall, 216 employees agreed to participate in the questionnaire survey and blood test, and replied with a signed consent form. Of these employees, 47 were excluded because of missing data in essential study parameters. An additional 21 employees reporting physical/mental disorders were excluded (see 'Covariates' section for detail), which resulted in a sample size of 148 participants at time 1. In April 2003 (Year 1, time 2), we followed-up 121 participants at the annual health examination (follow-up rate 81.8%). Of these employees, 16 were excluded because of missing data or reporting physical/mental disorders. In consequence, 105 healthy participants (67 men and 38 women) who provided complete sets of measurements in times 1 and 2 were enrolled in the analyses. Employees who participated in both times 1 and 2 and those who participated only in time 1 (non-participants) were similar except that non-participants were three years older, consumed more alcohol beverages, and showed slightly lower GHQ-28 score.

The study protocol was reviewed and approved by the Institutional Review Board of the National Institute of Occupational Safety and Health, Japan and by the Ethical Committee of the Kyushu University.

2.2. Measurements

2.2.1. Psychological distress

Psychological distress was assessed using the Japanese version (Iwata and Saito, 1992) of the 28-item GHQ (GHQ-28) (Goldberg and Hillier, 1979). We used Likert scale from 1 to 4, to calculate the score. If there are five or fewer missing responses on the GHQ-28, the total GHQ-28 score was calculated based on the following formula: "GHQ-28 score" = "sum of item scores answered (*X*)" × "28/*X*." The internal consistency (Cronbach's α) of the GHQ-28 in the present sample (n = 105) was 0.90 at time 1 and 0.88 at time 2.

2.2.2. Depressive symptoms

Depressive symptoms were measured using the Japanese version (Shima et al., 1985) of the CES-D (Radloff, 1977). The 20-item depressive symptom scale measures the level of depressive symptoms experienced in the past week. If there are three or less missing responses on the CES-D, the total CES-D score was calculated based on the following formula: "CES-D score" = "sum of item scores answered (*X*)" × "20/*X*." The Cronbach's α of the CES-D scale was 0.82 at time 1 and 0.87 at time 2.

2.2.3. Preparation of blood samples

Fasting blood samples were collected between 9.00 and 11.00 a.m. from participants to control for diurnal variations. Immunological parameters were determined by standard techniques, as described in detail elsewhere (Nakata et al., 2000; Nakata et al., 2010; Nakata et al., 2002). We determined counts of total leukocytes and total lymphocytes by an automated cell counter (Coulter Counter SP-VI, Coulter Electronics, Hialeah, FL).

2.2.4. Cell surface marker analysis

The following sets of monoclonal antibodies were used to perform four-color direct immunofluorescence surface-marker analysis: anti-CD45-FITC/anti-CD56-RD1/anti-CD19-ECD/anti-CD3-PC5. Anti-CD45-FITC antibody was used to identify and differentiate lymphocytes from non-lymphocytes and debris. A combination of Mouse IgG1-FITC/Mouse IgG1-RD1/Mouse IgG1-ECD/Mouse IgG1-PC5 was used as the negative control. All monoclonal antibodies were purchased from Beckman Coulter Inc, USA. We calculated the number in each lymphocyte subset by multiplying lymphocyte counts by the percentage of positive cells in each category, as determined by flow cytometer (EPICS XL, Beckman Coulter Inc, CA).

2.2.5. Cytotoxicity assay

A standard 4-hour Chromium-51 (^{51}Cr) release assay was used to determine NKCC with effector cells at an effector/target [E/T] cell ratio

of 20:1 (Pross et al., 1981). K562 was used as target cells and labeled with [^{51}Cr]-sodium chromate (New England Nuclear, Boston, Mass., USA) at 37 °C for an hour, washed and re-suspended at 2×10^5/ml in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% Fetal Calf Serum, 2 mM glutamine, 100 U/ml penicillin and 100 U/ml streptomycin. Labeled target cells were incubated with effector cells at an effector/target [E/T] cell ratio of 20:1 in U-bottomed 96-well plates at 37 °C for 4 h. Radioactivity in the supernatant was determined by a gamma counter. The assay was performed in quadruplicate. The percentage of specific lysis as cytotoxicity was determined according to the following formula: percentage of specific lysis = [(mean experimental cpm release − mean spontaneous cpm release)/(mean maximal cpm release − mean spontaneous cpm release)]. We chose the E/T cell ratio of 20:1 because a large study of Japanese population (n = 3625) identified that the differences between individuals' cytotoxic activity were most distinguishable (Imai et al., 2000). NKCC was measured at time 1 only.

2.2.6. Covariates

Covariates included sex, age (in years), education (in years), marital status (unmarried/married), smoking (number of cigarettes smoked per day), alcohol consumption (g ethanol/week), leisure-time physical activity, perceived daytime sleepiness, height, weight, typical work hours per day, self-reported illness, and regular medication usage.

Alcohol consumption was estimated by asking the usual amount of alcoholic drinks consumed per day multiplied by the number of occasions in a week that alcoholic drinks were consumed. We assessed leisure-time physical activity by calculating the energy expenditure of habitual physical exercise. We asked frequency, type, and length of physical exercise per month and converted these data to metabolic equivalents (METs) (Suzuki et al., 1998). Perceived daytime sleepiness was assessed using the Epworth Sleepiness Scale (ESS) score (Johns, 1991) translated into Japanese by a sleep expert (Tachibana, 2002). The ESS uses a self-administered questionnaire to measure sleep propensities in eight different real-life situations. The sum of eight individual scores (range, 0–24) yields the ESS score; higher score indicating greater sleepiness. Cronbach's α for the ESS was 0.73. Height (m) and weight (kg) were measured anthropometrically to assess body mass index (BMI), calculated as weight in kilograms divided by the square of height in meters. Regarding physical/psychological illness, participants were asked by an occupational health doctor/nurse if they had been diagnosed or treated for any of the following symptoms or disorders at the time of the study: hypertension, diabetes mellitus, depression, asthma, allergies, cancer, angina pectoris, cardiac infarction, gout, renal disease, colonic polyp, skin disease, anxiety disorders, musculoskeletal disorders, arrhythmia, cholelithiasis, kidney and urinary track diseases, liver disease, cerebrovascular disease, hyperlipidemia, gastric/duodenal ulcer, autonomic imbalance, or other diseases. If the subjects reported 'other diseases,' they were asked to specify the condition. As a result, participants with the following disorders were identified; hypertension (n = 13), hyperlipidemia (n = 10), diabetes mellitus (n = 4), gastric/duodenal ulcer (n = 3), depression (n = 2), asthma (n = 2), liver diseases (n = 2), autonomic imbalance (n = 2), anxiety disorders (n = 1), severe allergies (n = 1), and the common cold (n = 1); 20 participants reported 2+ disorders. In order to eliminate the potential effects of health status on immune parameters, we excluded all participants who reported the above disorders (n = 21) from the analyses. We also obtained data on the use of the following medications; aspirin (n = 17), β-blockers (n = 1), acetaminophen (n = 4), corticosteroids (n = 1), antidepressants (n = 1), and anxiolytic drugs (n = 1). All participants with self-reported illnesses as described above were eliminated, leaving only aspirin (n = 12) and acetaminophen (n = 4) users in the subsequent analyses; these users were categorized as medication users (yes/no) in the analyses.

2.3. Statistical analyses

Variables (age, alcohol consumption, BMI, ESS, GHQ-28, and CES-D scale score) with skewed distributions were logarithmically transformed to achieve a more normal distribution in values. Alcohol consumption, CES-D and ESS were scaled for a non-negative value by adding 1 for correlational analyses. The differences of psychological measures (GHQ-28 and CES-D) and immune markers between baseline (time 1) and year 1 (time 2) were examined by a paired-sample *t* test. Intercorrelations between psychological measures and immune markers were tested by the Pearson product-moment correlation coefficient.

Hierarchical multiple linear regression analysis was performed to test the association of psychological measures at time 1 with immune markers at time 2. Similarly, to test the opposite directionality, relationships between immune markers at time 1 and psychological measures at time 2 were analyzed. In step 1, we entered sex and age at time 1; step 1 was then adjusted for an immune variable (NK, T or B cells) or a psychological measure (GHQ-28 or CES-D scores) at time 1. Finally, in step 3, we adjusted for all potential confounders including step 2 variables and marital status, education, smoking, alcohol consumption, physical activity, ESS score, BMI, work hours, and medication usage. In the analytic process, we considered the interactions of sex × psychological measures or sex × immune variables but there were no significant ($p<.05$) interactions thus they were not incorporated into the further analyses.

The significance level for all statistical analyses was $p<0.05$ (two-tailed test). We analyzed the data using the Statistical Package for the Social Sciences version 15.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

Descriptive statistics for participants at times 1 and 2 are shown in Table 1. Roughly two-thirds were men with an average age of 40 years for

Table 1
Characteristics of study participants (n = 105) [a].

	Time 1 (baseline)	Time 2 (Year 1)	p[b]
	Mean ± SD or n (%)	Mean ± SD or n (%)	
Demographic and lifestyle variables:			
Sex, % men	67 (63.8)	67 (63.8)	–
Age (in years)	39.8 ± 10.7	40.8 ± 10.7	–
Education (in years)	15.3 ± 1.5	15.3 ± 1.5	–
Marital status, % married	70 (66.7)	70 (66.7)	–
Smoking (number of cigarettes smoked/day)	7.4 ± 12.2	7.0 ± 11.6	.347
Alcohol consumption (g ethanol/week)	121.6 ± 129.1	113.3 ± 117.0	.185
Leisure-time physical activity (METs/week)	5.0 ± 8.8	3.8 ± 5.8	.126
Daytime sleepiness (Epworth Sleepiness Scale) score	7.5 ± 3.8	6.9 ± 3.3	.026
BMI (kg/height (m)^2)	22.5 ± 2.9	22.4 ± 2.9	.843
Work hours/day	9.7 ± 2.0	9.6 ± 2.1	.672
Medication usage[c], % yes	16 (15.2)	16 (15.2)	–
Psychological measures:[d]			
GHQ-28 score	50.8 ± 10.3	50.2 ± 9.1	.665
CES-D score	11.9 ± 6.6	10.7 ± 7.3	.089
Immune markers:			
NK (CD3-CD56+) cells (cells/mm^3)	325 ± 214	197 ± 128	<.001
Total T (CD3 + CD56-) cells (cells/mm^3)	1151 ± 425	873 ± 258	<.001
B (CD19+) cells (cells/mm^3)	242 ± 144	167 ± 112	<.001
NKCC (% cytotoxicity)	51.5 ± 15.9	N/A	–

[a] Participants who reported immune-related disorders at times 1 and 2 were excluded to eliminate the potential effects of health status on immune parameters.
[b] Paired-sample *t* test.
[c] Use of either acetaminophen or aspirin.
[d] Negatively oriented.

total participants at baseline. The average age of education was 15 years (range 12–20); two-thirds were married and 15% of participants used acetaminophen or aspirin containing over-the-counter medications.

Except ESS score, no significant differences were found between times 1 and 2 with regard to smoking, alcohol consumption, physical activity, BMI, and work hours. Similarly, the GHQ-28 and CES-D scores between times 1 and 2 were not significantly different. In contrast, numbers of NK, T, and B cells were significantly smaller in time 2 compared to time 1.

Intercorrelations between psychological variables and immune markers at times 1 and 2 are shown in Table 2. Cross-sectional analyses revealed that GHQ-28 score was inversely associated with NK cells in both times 1 and 2; CES-D score was significantly associated with NK cells at time 1 only. NKCC was significantly associated with GHQ-28 score but not with CES-D score while T cells were inversely related to CES-D score but not with GHQ-28. NKCC was positively associated with NK cells at times 1 and 2.

Prospective analyses found that GHQ-28 and CES-D scores at time 1 were inversely related to NK cells at time 2; GHQ-28 and CES-D scores at time 2 were also inversely related to NK cells at time 1. In addition, CES-D scores at time 1 was inversely associated with T cells. All lymphocyte subset counts were highly correlated between times 1 and 2.

The results of the hierarchical multiple linear regression analysis predicting time 2 immune variables by time 1 psychological variables are shown in Table 3 and summarized in Fig. 1A. Both GHQ-28 and CES-D scores were consistently associated with a decrease of NK cells, while CES-D score at time 1 were inversely related to T cells in step 1 only. B cells at time 2 were not associated with GHQ-28 and CES-D scores.

Regression analysis predicting time 2 psychological variables by time 1 immune variables are shown in Table 4 and Fig. 1B. In step 1, NK cells at time 1 were associated with time 2 GHQ-28 scores but the association disappeared once time 1 GHQ-28 score and/or other covariates were controlled for. T and B cells at time 1 did not predict GHQ-28 and CES-D scores at time 2.

4. Discussion

The current study investigated the prospective and bi-directional associations of psychological distress and depressive symptoms with cell-mediated immunity among 105 healthy individuals and the following findings were obtained. First, baseline values of psychological measures were associated with subsequent reduction of NK cells but not with T or B cells (Table 3, Fig. 1A). Second, baseline NK (as well as T or B) cells and NKCC did not predict subsequent levels of psychological measures (Table 4, Fig. 1B). Third, a moderate correlation between NK cell counts and NKCC indicates that not only quantity but also function of NK cells might be impaired by psychological distress/depressive symptoms. Due to the fact that the study participants were healthy individuals without extreme and apparent psychological disturbances, we conclude that even a mild to moderate level of psychological distress/depressive symptoms may impair NK cell immunity but not the other way round.

Lack of bi-directional relationships between psychological distress/depressive symptoms and cellular immune markers suggests that there may be no feedback loop mechanism that was proposed in the depression–inflammation relationship (Howren et al., 2009; Matthews et al., 2010). This finding implies that suppression of NK cell immunity by psychological distress or depressive symptoms may be mediated by inflammatory processes and thus has no direct relationship between the two. Alternatively, suppression of NK cells and elevated inflammatory markers by psychological distress and depressive symptoms may be unrelated processes occurring separately (Blume et al., 2011), because a previous cross-sectional study found a dissociation of inflammatory markers (IL-6, soluble IL-2R, and acute phase proteins) and NKCC in patients with major depressive disorder (Pike and Irwin, 2006). It should be noted, however, that this assumption should be tested in other clinical settings with multiple follow-up evaluations measuring both cellular and inflammatory markers.

The association between psychological distress and depressive symptoms and NK cells may be related to CVD and certain type of cancer because NK cells appear to play a major role in killing virally infected cells as well as cancerous cells (Whiteside and Herberman, 1994). It has been suggested that chronic infections contribute to inflammatory activity and hence to the development of CVD (Clays et al., 2005). Meanwhile, impaired NK cell immunity by psychological distress/depressive symptoms may be associated with cancer development and progression in the long run. With regard to the relationship between NKCC and cancer incidence, a prospective study of 3625 residents of Japanese general population indicated

Table 2
Pearson correlation matrix for psychological measures and immune variables (n = 105).

Variable	1		2		3		4		5		6		7		8		9		10	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Time 1																				
1. GHQ-28[a,b]	–	–																		
2. CES-D[a,b]	**.584**	**<.001**	–	–																
3. NK (CD3-CD56+) cells (cells/mm^3)	**−.378**	**<.001**	**−.253**	**.009**	–	–														
4. T (CD3 + CD56-) cells (cells/mm^3)	−.063	.526	**−.211**	**.031**	−.043	.666	–	–												
5. B (CD19+) cells (cells/mm^3)	.076	.440	.063	.523	.077	.437	**.326**	**<.001**	–	–										
6. NKCC	**−.240**	**.014**	−.160	.102	**.523**	**<.001**	−.111	.260	−.124	.207	–	–								
Time 2																				
7. GHQ-28[a,b]	**.590**	**<.001**	**.445**	**<.001**	**−.249**	**.010**	−.103	.296	−.011	.913	−.087	.377	–	–						
8. CES-D[a,b]	**.421**	**<.001**	**.518**	**<.001**	**−.194**	**.047**	−.167	.089	.059	.551	−.065	.513	**.596**	**<.001**	–	–				
9. NK (CD3-CD56+) cells (cells/mm^3)	**−.382**	**<.001**	**−.295**	**.002**	**.520**	**<.001**	.088	.371	−.012	.906	**.252**	**.010**	**−.257**	**.008**	−.105	.288	–	–		
10. T (CD3 + CD56-) cells (cells/mm^3)	−.059	.548	**−.251**	**.010**	**.243**	**.012**	**.715**	**<.001**	**.337**	**<.001**	.026	.795	−.090	.363	−.175	.074	**.207**	**.034**	–	–
11. B (CD19+) cells (cells/mm^3)	.030	.760	−.058	.558	.018	.852	**.465**	**<.001**	**.734**	**<.001**	−.098	.318	−.052	.598	.000	1.00	.068	.488	**.348**	**<.001**

Bold type indicates variables at $p<.05$ level.
[a] Negatively oriented.
[b] Log-transformed.

Table 3
Summary of hierarchical multiple linear regression analysis for predicting immune markers at time 2 and psychological measures at time 1 (n = 105).

Immune markers (dependent variable)	Step 1[a]						Step 2[b]						Step 3[c]					
	NK cells (time 2)		T cells (time 2)		B cells (time 2)		NK cells (time 2)		T cells (time 2)		B cells (time 2)		NK cells (time 2)		T cells (time 2)		B cells (time 2)	
	β^d	p	β^d	p	β^d	p	β^d	p	β^d	p	β^d	p	β^d	p	β^d	p	β^d	p
GHQ-28 score[e,f] (Time 1)	**−.374**	**<.001**	−.044	.653	.048	.629	**−.204**	**.026**	−.005	.946	−.023	.732	**−.221**	**.030**	.079	.293	−.087	.264
CES-D score[e,f] (Time 1)	**−.279**	**.004**	**−.229**	**.019**	−.032	.743	−.166	.057	−.090	.200	−.095	.165	**−.177**	**.043**	−.059	.414	−.119	.099

Bold type indicates variables at $p<.05$ level.
[a] Adjusted for sex and age at time 1.
[b] Adjusted for sex, age, and immune variable (NK, T or B cells) at time 1.
[c] Adjusted for sex, age, immune variable (NK, T or B cells), education, marital status, smoking, alcohol consumption, physical activity, BMI, medication usage, work hours, and ESS score at time 1.
[d] Standardized regression coefficients for each variable's unique contribution.
[e] Negatively oriented.
[f] Log-transformed.

that those who had the lowest thirds of NKCC level at baseline had 1.59 times higher risk of cancer incidence (all-site) than the highest thirds of NKCC level after 11 years of observation; the lowest thirds even had a 1.69 times higher risk than the medium NKCC level (Imai et al., 2000). However, our speculation that psychological distress/depressive symptoms is connected to suppression of NK cells leading to future CVD/cancer needs to be confirmed by well-designed prospective studies in the future.

No significant associations between psychological distress/depressive symptoms and T or B cells were found in this study. As stated earlier, function of T (and in lesser degree B) cells are reduced by psychological distress and depressive symptoms (Herbert and Cohen,

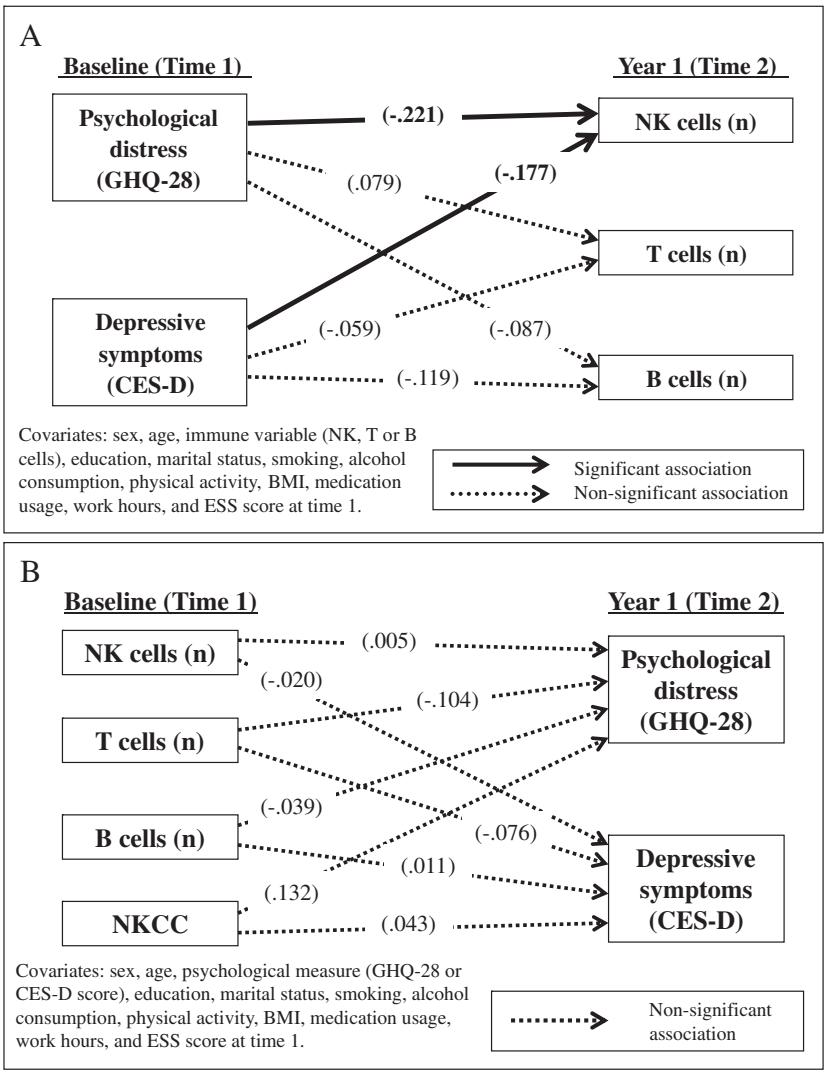

Fig. 1. Longitudinal association between psychological measures and immune markers. (A) illustrates baseline psychological measures (GHQ-28 and CES-D) predicting circulating immune cells at Year 1. (B) depicts baseline immune markers predicting psychological measures (GHQ-28 and CES-D) at Year 1. Values are standardized regression coefficients.

Table 4
Summary of hierarchical multiple linear regression analysis for predicting psychological measures at time 2 and immune markers at time 1 (n = 105).

Mental health measures (dependent variable)	Step 1[a]				Step 2[b]				Step 3[c]			
	GHQ-28 score[d,e] (time 2)		CES-D score[d,e] (time 2)		GHQ-28 score[d,e] (time 2)		CES-D score[d,e] (time 2)		GHQ-28 score[d,e] (time 2)		CES-D score[d,e] (time 2)	
	β^f	p	β^f	p	β^f	p	β^f	p	β^f	p	β^f	p
NK cells (time 1)	**−.238**	**.017**	−.179	.073	−.001	.990	−.060	.504	.005	.958	−.020	.841
T cells (time 1)	−.096	.333	−.155	.115	−.063	.434	−.056	.524	−.104	.268	−.076	.456
B cells (time 1)	−.002	.983	.083	.407	−.062	.444	.040	.650	−.039	.694	.011	.920
NKCC (time 1)	−.071	.487	−.056	.585	.104	.226	.036	.494	.132	.172	.043	.669

Bold type indicates variables at $p<.05$ level.
[a] Adjusted for sex and age at time 1.
[b] Adjusted for sex, age, and psychological measure (GHQ-28 or CES-D scores) at time 1.
[c] Adjusted for sex, age, psychological measure (GHQ-28 or CES-D score), education, marital status, smoking, alcohol consumption, physical activity, BMI, medication usage, work hours, and ESS score at time 1.
[d] Negatively oriented.
[e] Log-transformed.
[f] Standardized regression coefficients for each variable's unique contribution.

1993; Weisse, 1992; Zorrilla et al., 2001). It may be speculated that psychological distress/depressive symptoms are reliably associated with function but not quantity.

Although our study has several strengths, including the prospective study design, adjustment of multiple confounders, and using two standardized screening instruments, our study has potential weaknesses. First, sample size was not large and we included only one follow-up occasion with intervals been one year. It would be ideal to assess psychological and immune measures multiple (3+) times to enhance accuracy of causal inference. Second, the ranges of psychological scores were somewhat restricted, which might have contributed to underestimation of effect size. Third, we could not obtain data on menstrual phase or oral contraceptive use in women, which might have some effects on immunological outcomes. Fourth, we did not simultaneously measure inflammatory markers as well as NKCC at time 2, which limit the interpretation of our findings. And finally, although we adjusted for a variety of confounders, we could not exclude the possibility that unadjusted factors, i.e., personality traits and genetic components, as well as unknown factors may have affected our findings.

In conclusion, this study examined the longitudinal association of psychological distress and depressive symptoms with cellular immunity in a sample of 105 healthy Japanese employees. The results suggested that psychological distress and depressive symptoms may impair NK cell immunity but reduced NK cells may not lead to psychological distress and depressive symptoms. Although the precise mechanisms and pathways underlying the observed associations have yet to be determined, the findings of the present study provides some support for the biological plausibility of the relationships between psychological distress, depressive symptoms and immune-related health.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the US National Institute for Occupational Safety and Health.

References

Amati, M., Tomasetti, M., Ciuccarelli, M., Mariotti, L., Tarquini, L.M., Bracci, M., Baldassari, M., Balducci, C., Alleva, R., Borghi, B., Mocchegiani, E., Copertaro, A., Santarelli, L., 2010. Relationship of job satisfaction, psychological distress and stress-related biological parameters among healthy nurses: a longitudinal study. J. Occup. Health. 52, 31–38.

Bauer, M.E., Gauer, G.J., Luz, C., Silveira, R.O., Nardi, N.B., von Muhlen, C.A., 1995. Evaluation of immune parameters in depressed patients. Life Sci. 57, 665–674.

Bjerkeset, O., Romild, U., Smith, G.D., Hveem, K., 2011. The associations of high levels of C-reactive protein with depression and myocardial infarction in 9258 women and men from the HUNT population study. Psychol. Med. 41, 345–352.

Blume, J., Douglas, S.D., Evans, D.L., 2011. Immune suppression and immune activation in depression. Brain Behav. Immun. 25, 221–229.

Clays, E., De Bacquer, D., Delanghe, J., Kittel, F., Van Renterghem, L., De Backer, G., 2005. Associations between dimensions of job stress and biomarkers of inflammation and infection. J. Occup. Environ. Med. 47, 878–883.

Cuijpers, P., Smit, F., 2002. Excess mortality in depression: a meta-analysis of community studies. J. Affect. Disord. 72, 227–236.

Evans, D.L., Folds, J.D., Petitto, J.M., Golden, R.N., Pedersen, C.A., Corrigan, M., Gilmore, J.H., Silva, S.G., Quade, D., Ozer, H., 1992. Circulating natural killer cell phenotypes in men and women with major depression. Relation to cytotoxic activity and severity of depression. Arch. Gen. Psychiatry 49, 388–395.

Evans, D.L., Ten Have, T.R., Douglas, S.D., Gettes, D.R., Morrison, M., Chiappini, M.S., Brinker-Spence, P., Job, C., Mercer, D.E., Wang, Y.L., Cruess, D., Dube, B., Dalen, E.A., Brown, T., Bauer, R., Petitto, J.M., 2002. Association of depression with viral load, CD8 T lymphocytes, and natural killer cells in women with HIV infection. Am. J. Psychiatry 159, 1752–1759.

Fortes, C., Farchi, S., Forastiere, F., Agabiti, N., Pacifici, R., Zuccaro, P., Perucci, C.A., Ebrahim, S., 2003. Depressive symptoms lead to impaired cellular immune response. Psychother. Psychosom. 72, 253–260.

Frank, M.G., Wieseler Frank, J.L., Hendricks, S.E., Burke, W.J., Johnson, D.R., 2002. Age at onset of major depressive disorder predicts reductions in NK cell number and activity. J. Affect. Disord. 71, 159–167.

Gimeno, D., Kivimaki, M., Brunner, E.J., Elovainio, M., De Vogli, R., Steptoe, A., Kumari, M., Lowe, G.D., Rumley, A., Marmot, M.G., Ferrie, J.E., 2009. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. Psychol. Med. 39, 413–423.

Goldberg, D.P., Hillier, V.F., 1979. A scaled version of the General Health Questionnaire. Psychol. Med. 9, 139–145.

Herbert, T.B., Cohen, S., 1993. Depression and immunity: a meta-analytic review. Psychol. Bull. 113, 472–486.

Howren, M.B., Lamkin, D.M., Suls, J., 2009. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom. Med. 71, 171–186.

Imai, K., Matsuyama, S., Miyake, S., Suga, K., Nakachi, K., 2000. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 356, 1795–1799.

Irwin, M.R., Miller, A.H., 2007. Depressive disorders and immunity: 20 years of progress and discovery. Brain Behav. Immun. 21, 374–383.

Irwin, M., Lacher, U., Caldwell, C., 1992. Depression and reduced natural killer cytotoxicity: a longitudinal study of depressed patients and control subjects. Psychol. Med. 22, 1045–1050.

Iwata, N., Saito, K., 1992. The factor structure of the 28-item General Health Questionnaire when used in Japanese early adolescents and adult employees: age- and cross-cultural comparisons. Eur. Arch. Psychiatry Clin. Neurosci. 242, 172–178.

Johns, M.W., 1991. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14, 540–545.

Jung, W., Irwin, M., 1999. Reduction of natural killer cytotoxic activity in major depression: interaction between depression and cigarette smoking. Psychosom. Med. 61, 263–270.

Leserman, J., Petitto, J.M., Perkins, D.O., Folds, J.D., Golden, R.N., Evans, D.L., 1997. Severe stress, depressive symptoms, and changes in lymphocyte subsets in human immunodeficiency virus-infected men. A 2-year follow-up study. Arch. Gen. Psychiatry 54, 279–285.

Matthews, K.A., Schott, L.L., Bromberger, J.T., Cyranowski, J.M., Everson-Rose, S.A., Sowers, M., 2010. Are there bi-directional associations between depressive symptoms and C-reactive protein in mid-life women? Brain Behav. Immun. 24, 96–101.

McGuire, L., Kiecolt-Glaser, J.K., Glaser, R., 2002. Depressive symptoms and lymphocyte proliferation in older adults. J. Abnorm. Psychol. 111, 192–197.

Nakata, A., Araki, S., Tanigawa, T., Miki, A., Sakurai, S., Kawakami, N., Yokoyama, K., Yokoyama, M., 2000. Decrease of suppressor-inducer (CD4+ CD45RA) T lymphocytes and increase of serum immunoglobulin G due to perceived job stress in

Japanese nuclear electric power plant workers. J. Occup. Environ. Med. 42, 143–150.
Nakata, A., Tanigawa, T., Fujioka, Y., Kitamura, F., Iso, H., Shimamoto, T., 2002. Association of low job control with a decrease in memory (CD4+ CD45RO+) T lymphocytes in Japanese middle-aged male workers in an electric power plant. Ind. Health 40, 142–148.
Nakata, A., Takahashi, M., Irie, M., Swanson, N.G., 2010. Job satisfaction is associated with elevated natural killer cell immunity among healthy white-collar employees. Brain Behav. Immun. 24, 1268–1275.
Nicholson, A., Kuper, H., Hemingway, H., 2006. Depression as an aetiologic and prognostic factor in coronary heart disease: a meta-analysis of 6362 events among 146,538 participants in 54 observational studies. Eur. Heart J. 27, 2763–2774.
Park, E.J., Lee, J.H., Chae, J.H., Lee, K.H., Han, S.I., Jeon, Y.W., 2006. Natural killer T cells in patients with major depressive disorder. Psychiatry Res. 144, 237–239.
Pike, J.L., Irwin, M.R., 2006. Dissociation of inflammatory markers and natural killer cell activity in major depressive disorder. Brain Behav. Immun. 20, 169–174.
Pinquart, M., Duberstein, P.R., 2010. Depression and cancer mortality: a meta-analysis. Psychol. Med. 40, 1797–1810.
Pross, H.F., Baines, M.G., Rubin, P., Shragge, P., Patterson, M.S., 1981. Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantitation of natural killer cell activity. J. Clin. Immunol. 1, 51–63.
Radloff, L., 1977. The CES-D Scale: a self-reported depression scale for research in general population. Appl. Psychol. Meas. 1, 385–401.
Schleifer, S.J., Keller, S.E., Bond, R.N., Cohen, J., Stein, M., 1989. Major depressive disorder and immunity. Role of age, sex, severity, and hospitalization. Arch. Gen. Psychiatry 46, 81–87.
Segerstrom, S.C., Miller, G.E., 2004. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol. Bull. 130, 601–630.
Shima, S., Shikano, T., Kitamura, T., Asai, M., 1985. A new self-rating scale for depression [in Japanese]. Clin. Psychiatry 27, 717–723.
Steptoe, A., Kunz-Ebrecht, S.R., Owen, N., 2003. Lack of association between depressive symptoms and markers of immune and vascular inflammation in middle-aged men and women. Psychol. Med. 33, 667–674.
Stewart, J.C., Rand, K.L., Muldoon, M.F., Kamarck, T.W., 2009. A prospective evaluation of the directionality of the depression–inflammation relationship. Brain Behav. Immun. 23, 936–944.
Suzuki, I., Kawakami, N., Shimizu, H., 1998. Reliability and validity of a questionnaire for assessment of energy expenditure and physical activity in epidemiological studies. J. Epidemiol. 8, 152–159.
Tachibana, N., 2002. Insomnia and excessive sleepiness. In: Imura, H. (Ed.), Simple Internal Medicine. Bunkodo, Tokyo, pp. 1094–1106.
Tsuboi, H., Kawamura, N., Hori, R., Kobayashi, F., Iwasaki, Y., Takeuchi, H., Fukino, O., 2005. Depressive symptoms and life satisfaction in elderly women are associated with natural killer cell number and cytotoxicity. Int. J. Behav. Med. 12, 236–243.
Weisse, C.S., 1992. Depression and immunocompetence: a review of the literature. Psychol. Bull. 111, 475–489.
Whiteside, T.L., Herberman, R.B., 1994. Role of human natural killer cells in health and disease. Clin. Diagn. Lab. Immunol. 1, 125–133.
WHO, 2008. The Global Burden of Disease: 2004 Update. , p. 51.
Zorrilla, E.P., Luborsky, L., McKay, J.R., Rosenthal, R., Houldin, A., Tax, A., McCorkle, R., Seligman, D.A., Schmidt, K., 2001. The relationship of depression and stressors to immunological assays: a meta-analytic review. Brain Behav. Immun. 15, 199–226.