Lipopolysaccharide-stimulated intracellular cytokines and depressive symptoms in community-dwelling older adults

Joshua Hyong-Jin Cho[1]*
https://orcid.org/0000-0002-8629-8500

Sara M. Shu[2]*

Ariya Mahbod[1]

Michael R. Irwin[1]
https://orcid.org/0000-0002-1502-8431

[1] *Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience and Human Behavior, Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, California, USA.*
[2] *Western University of Health Sciences, Pomona, California, USA.*
**These authors equally contributed to this work.*

Received: 4/17/2019 – **Accepted:** 5/9/2019
DOI: 10.1590/0101-60830000000212

Abstract

Background: Inflammation is involved in the pathophysiology of depression, and circulating inflammatory cytokines have been associated with depressive symptoms. However, measuring circulating cytokines have inherent methodological limitations. *In vitro* lipopolysaccharide (LPS)-stimulated intracellular cytokines (ICCs) overcome these limitations. Furthermore, because psychosocial and physiological stressors activate inflammatory responses and LPS-stimulated ICCs reflect the inflammatory responsivity of monocytes to such stressors, ICCs may reflect individual stress responsivity. **Methods:** This cross-sectional study examined whether LPS-stimulated expression of ICCs in peripheral blood mononuclear cells (PBMCs) is a sensitive inflammation measure correlated with depressive symptoms in 180 community-dwelling older adults. We tested correlations of not only intracellular but also circulating inflammatory markers with depressive symptoms assessed using the 10-item Center for Epidemiological Studies Depression Scale (CES-D). Intracellular markers included expression of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and both in PBMCs. Circulating markers included IL-6, TNF-α, and C-reactive protein (CRP) in plasma. **Results:** None of the correlations were statistically significant. However, in contrast to circulating markers, the correlations of ICCs were consistently in the expected direction, i.e., higher ICC expression correlating with higher depression severity. **Discussion:** Despite the non-significant findings, further research is required for the evaluation of LPS-stimulated ICC expression as biomarkers of depressive symptoms.

Cho JH et al. / Arch Clin Psychiatry. 2019;46(5):137-40

Keywords: Inflammation, lipopolysaccharide, intracellular cytokines, depressive symptoms, older adults.

Introduction

Currently available antidepressants are mostly based on the monoamine hypothesis and have their limitations. Thus, exploring novel pathophysiology of depression is important for identification of biomarkers of depression and targeted treatment[1]. According to the inflammatory hypothesis of depression, proinflammatory cytokines produced by the innate immune response leads to depressive symptoms[2]. Meta-analyses have shown concentrations of circulating interleukin (IL)-6, tumor necrosis factor (TNF)-α, soluble TNF receptor 2 (sTNFR2), and C-reactive protein (CRP) to be elevated in depressed patients compared to controls[3-7]. Current research regarding inflammatory markers and depression involves measurement of circulating cytokines (and CRP) in plasma or serum. This detection method comes with inherent limitations such as difficulty in detecting protein-bound cytokines, short half-life of cytokines, and interference of biological inhibitors with immunodetection[8]. These issues possibly have led to some conflicting results in the literature including negative (i.e., inverse) correlations between circulating inflammatory markers and depressive symptoms[9-11] and the extensive heterogeneity across individual studies included in meta-analyses[12,13]. Therefore, more sensitive biomarkers that overcome the limitations of circulating inflammatory markers are needed to characterize inflammation-associated depressive states.

One possible solution is the direct detection of intracellular cytokines (ICCs) after lipopolysaccharide (LPS) stimulation *in vitro*. Such methodology is advantageous as it is able to directly detect levels of ICCs without extracellular background. Stimulated ICC method has shown positive associations between severity of depression and monocyte-associated proinflammatory cytokines in healthy younger adults[14,15]. Furthermore, because psychosocial and physiological stressors activate inflammatory responses[16] and LPS-stimulated ICC expression reflects the inflammatory responsivity of cells to such stressors, this novel method may reflect individual stress responsivity.[17]. To our knowledge, no study has yet investigated the association between ICCs and depressive symptoms in a population of community-dwelling older adults. About 3% of the general elderly population meet the criteria for major depression[18], and it is estimated that 15% of community-dwelling older adults have clinically significant depressive symptoms[19]. The role of inflammation is particularly important in older adults as human aging is characterized by a chronic low-grade inflammation, a phenomenon named as "inflammaging", which increases the risk for numerous physical and mental illnesses[20].

This study aimed to directly compare circulating inflammatory marker levels in plasma and ICC levels in LPS-stimulated mononuclear cells as correlates of depressive symptoms in community-dwelling older adults. This novel method using ICCs may further advance the examination of inflammatory profiles and their association with depressive symptoms. Such knowledge will be valuable in early diagnosis, treatment, and possibly prevention of depression.

Address for correspondence: Joshua H. Cho. 300 UCLA Medical Plaza, Suite 3200A – 90095 – Los Angeles, California. E-mail: hjcho@mednet.ucla.edu

Methods

Participants

This study was a secondary analysis of data and blood samples from a cross-sectional study designed to assess the associations between social isolation and health outcomes such as depression, insomnia and fatigue among community-dwelling adults aged 60 years and above in Los Angeles. The study received oversight and approval from the UCLA Institutional Review Board (IRB#11-000656). As previously described[21], age-targeted sampling methods were used to complete a telephone survey of a community sample of older adults (N = 2,541). Of this survey sample, 552 older adults were randomly invited to undergo an in-person interview with blood sampling; 287 consented to participate. After eligibility screening and blood sample assays, the complete data on depressive symptoms and ICCs were available in 180 participants, who comprised the sample of the current analysis.

Procedures

Depressive symptoms were measured using the 10-item Center for Epidemiological Studies Depression Scale (CES-D)[22]. Scores ≥ 4 are indicative of clinically significant depressive symptoms. For each subject included in the current analysis, a blood sample was taken, and peripheral blood mononuclear cells (PBMCs) were isolated from these whole blood samples. Cells were stimulated with 100 pg/mL LPS and incubated for four hours. Percentages of mononuclear cell expression of ICCs (IL-6 and TNF-α) were measured using flow cytometry. Circulating inflammatory markers in plasma including sTNFR2, IL-6, and CRP were also measured.

Statistical analysis

Non-normally distributed variables, such as circulating inflammatory markers, were natural log transformed. Sample characteristics and their associations with depressive symptoms were examined using t-test or chi-square test. Associations between inflammatory markers and depressive symptoms were analyzed via multivariate linear regression with standardized regression coefficient, β. Results were adjusted for covariates: age, sex, race, ethnicity, education, and body-mass index (BMI).

Results

Sample characteristics

Sample characteristics and their association with CES-D scores are shown in Table 1 (N = 180; aged 60-88, mean 72 years; 48.9% female). Depressive symptoms were largely mild with 84.4% of participants scoring below the CES-D cutoff of 4. Participants had slightly higher CRP levels (median 3.2 mg/L) compared to the US older adult population (median 2.9 mg/L)[23].

Inflammatory markers and depressive symptoms

There was no statistically significant association between depressive symptoms and ICCs: TNF-α (adjusted β = 0.040, p = 0.57), IL-6 (adjusted β = 0.082, p = 0.25), TNF-α+IL-6 (adjusted β = 0.078, p = 0.27). No statistically significant association was observed either between depressive symptoms and circulating inflammatory markers: sTNFR2 (adjusted β = -0.022, p = 0.79), IL-6 (adjusted β = -0.089, p = 0.26), CRP (adjusted β = -0.13, p = 0.09). Of note, the correlations between ICCs and depressive symptoms were non-significantly *positive*, i.e., at least in the expected direction of higher ICC expression correlating with higher depressive symptom severity. However, the correlations between circulating inflammatory markers and depressive symptoms were non-significantly *negative (i.e., inverse)* (Figure 1).

Discussion

To our knowledge, this study was the first to assess LPS-stimulated ICCs in relation to depressive symptoms in community-dwelling older adults, let alone to directly compare ICCs and circulating cytokines. However, neither ICCs nor circulating inflammatory markers were significantly associated with depressive symptoms in our sample of 180 older adults. Although non-significant, the correlations of ICCs with depressive symptoms were at least in the expected direction, i.e., positive correlations. However, the non-significant correlations between circulating markers and depressive symptoms were in an unexpected direction as higher levels correlated with lower depressive symptom severity, i.e., negative correlations.

Previous studies have almost exclusively measured circulating cytokines in association with depression; and several of them – conducted in different contexts including pregnancy[10], malignancies[11], and major depression[9] – have shown negative correlations between circulating cytokines and depressive symptoms. While few studies have used LPS-stimulated ICCs in relation to depression or depressive symptoms, the existing studies have shown positive correlations between ICCs and depressive symptoms in healthy younger volunteers[14,15].

Psychosocial and physiological stressors activate inflammatory responses[16]. Since *in vitro* LPS-stimulated ICC expression reflects the inflammatory responsivity of monocytes to such stressors, this novel method may be more appropriate as a marker of individual stress responsivity, a key risk factor for depression development[17], and therefore as a sensitive biomarker of depression. Furthermore, this approach may overcome the limitations of using circulating cytokines including difficulty detecting protein-bound cytokines, short half-life of cytokines, and interference of biological inhibitors with immunodetection[8]. Further development of such a sensitive and advantageous biomarker may certainly contribute to the personalized psychiatry. Unfortunately, the current study failed to convincingly demonstrate the superiority of ICCs compared to circulating cytokines, although some biological coherence was noted in the directionality of associations between ICCs and depressive symptoms (but not between circulating markers and depressive symptoms).

The following limitations should be considered. This was a cross-sectional study and no temporal relationship could be established. For a community-based non-clinical population, the sample size was somewhat small, likely reducing statistical power. Sample characteristics revealed a relatively healthy population with only mild depressive symptoms. This could explain the weak associations in our study. Moreover, we utilized the 10-item CES-D[22], which is a simplified version of the original CES-D. Although its reliability statistics have

Table 1. Characteristics of 180 participants according to the 10-item CES-D cutoff

Variable	CES-D < 4 (N = 152)	CES-D ≥ 4 (N = 28)	P-value
Age, years (SD)	72.8 (7.6)	66.4 (6.9)	< 0.001
Sex, female	46.7%	57.1%	0.31
Ethnicity, non-White	11.2%	32.1%	0.004
Education, years (SD)	15.7 (1.6)	15.1 (1.9)	0.08
Body-mass index (SD)	25.1 (3.7)	26.1 (2.9)	0.15
Charlson Comorbidity Index (SD)	4.7 (2.3)	4.2 (2.3)	0.25

CES-D: Center for Epidemiological Studies Depression scale; SD: standard deviation.

been comparable to the original CES-D, a more comprehensive and sensitive measure of depressive symptoms may be needed.

Further research is required for the evaluation of LPS-stimulated ICCs as novel biomarkers of depression, possibly with a larger community sample or a clinical sample with higher levels of depressive symptoms and/or systemic inflammation.

Acknowledgements

This research was funded by National Institutes of Health R01AG034588 (MRI). Additionally, the first author (JHC) was supported by K23AG049085 and R21MH113915. There is no conflict of interest to declare.

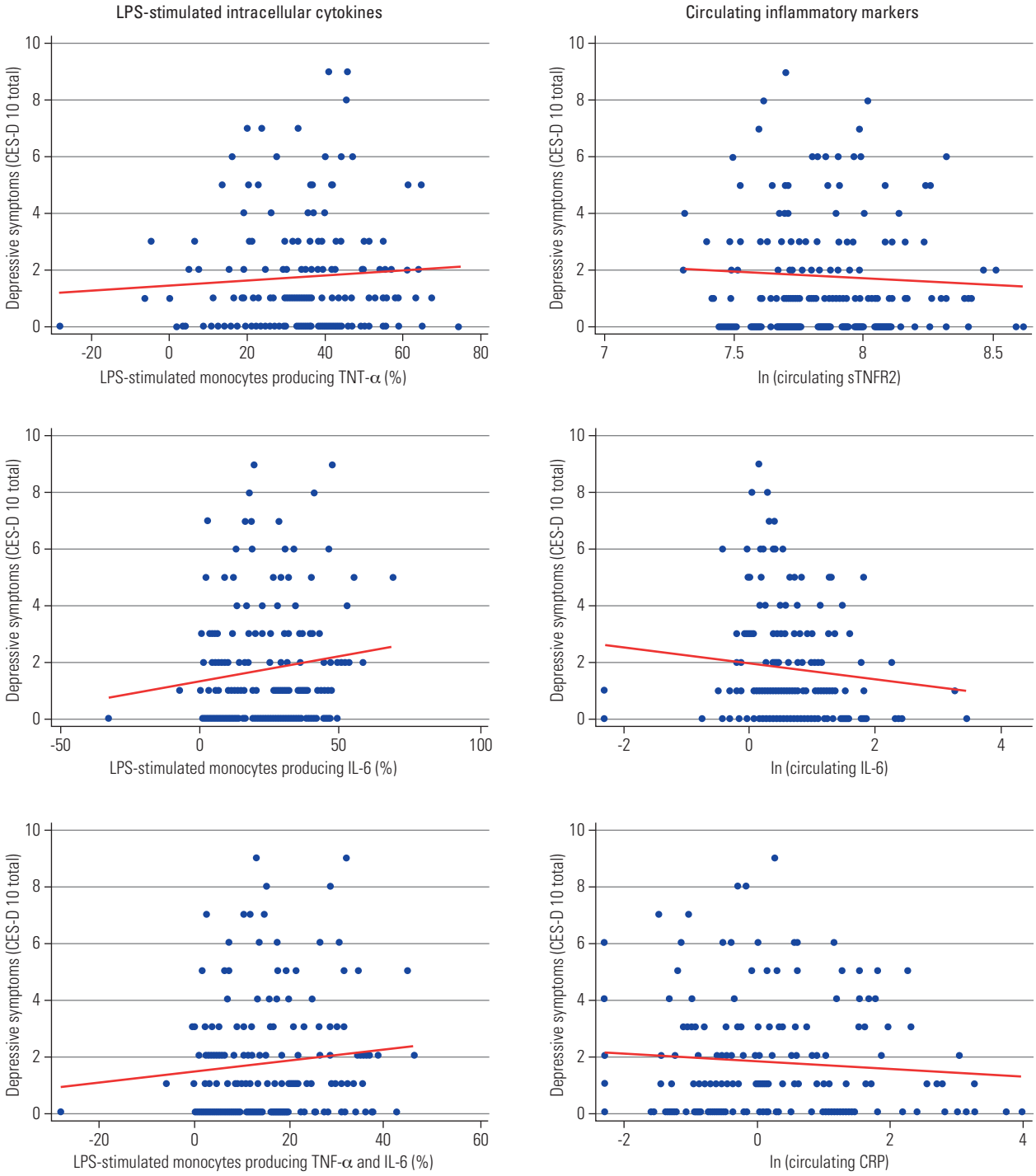

Figure 1. Correlations between inflammatory markers (LPS-stimulated vs. circulating) and depressive symptoms (CES-D) represented by scatter plots and linear fit lines.

LPS: lipopolysaccharide; CES-D: Center for Epidemiological Studies Depression scale; ln: log natural; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; sTNFR2: soluble TNF receptor; CRP: C-reactive protein.

References

1. Lang UE, Borgwardt S. Molecular mechanisms of depression: perspectives on new treatment strategies. Cell Physiol Biochem. 2013;31(6):761-77.
2. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev. 2008;9(1):46-56.
3. Howren MB, Lamkin DM, Suls J. Associations of depression with C--reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med. 2009;71(2):171-86.
4. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. Biol Psychiatry. 2010;67(5):446-57.
5. Liu Y, Ho RC, Mak A. Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta--regression. J Affect Disord. 2012;139(3):230-9.
6. Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, et al. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. Acta Psychiatr Scand. 2017;135(5):373-87.
7. Smith KJ, Au B, Ollis L, Schmitz N. The association between C-reactive protein, Interleukin-6 and depression among older adults in the community: A systematic review and meta-analysis. Exp Gerontol. 2018;102:109-32.
8. Wadhwa M, Thorpe R. Cytokine immunoassays: recommendations for standardisation, calibration and validation. J Immunol Methods. 1998;219(1-2):1-5.
9. Schmidt FM, Schröder T, Kirkby KC, Sander C, Suslow T, Holdt LM, et al. Pro-and anti-inflammatory cytokines, but not CRP, are inversely correlated with severity and symptoms of major depression. Psychiatry Res. 2016;239:85-91.
10. Shelton MM, Schminkey DL, Groer MW. Relationships among prenatal depression, plasma cortisol, and inflammatory cytokines. Biol Res Nurs. 2015;17(3):295-302.
11. Kim SY, Kim JM, Kim SW, Shin IS, Park MH, Yoon JH, et al. Associations between plasma cytokines and depressive mood in patients with breast cancer. Int J Psychiatry Med. 2012;43(1):1-17.
12. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimäki M. Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. Brain Behav Immun. 2015;49:206-15.
13. Hiles SA, Baker AL, de Malmanche T, Attia J. A meta-analysis of differences in IL-6 and IL-10 between people with and without depression: exploring the causes of heterogeneity. Brain Behav Immun. 2012;26(7):1180-8.
14. Suarez EC, Krishnan RR, Lewis JG. The relation of severity of depressive symptoms to monocyte-associated proinflammatory cytokines and chemokines in apparently healthy men. Psychosom Med. 2003;65(3):362-8.
15. Suarez EC, Lewis JG, Krishnan RR, Young KH. Enhanced expression of cytokines and chemokines by blood monocytes to in vitro lipopolysaccharide stimulation are associated with hostility and severity of depressive symptoms in healthy women. Psychoneuroendocrinology. 2004;29(9):1119-28.
16. Black PH. Stress and the inflammatory response: a review of neurogenic inflammation. Brain Behav Immun. 2002;16(6):622-53.
17. Bale TL. Stress sensitivity and the development of affective disorders. Horm Behav.. 2006;50(4):529-33.
18. Cole MG, Yaffe MJ. Pathway to psychiatric care of the elderly with depression. Int J Geriatr Psychiatry. 1996;11(2):157-61.
19. Fiske A, Wetherell JL, Gatz M. Depression in older adults. Annu Rev Clin Psychol. 2009;5:363-89.
20. Franceschi C, Campisi J. Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases. The Journals of Gerontology: Series A. 2014;69(Suppl_1):S4-S9.
21. Cho JH, Olmstead R, Choi H, Carrillo C, Seeman TE, Irwin MR. Associations of objective versus subjective social isolation with sleep disturbance, depression, and fatigue in community-dwelling older adults. Aging Ment Health. 2018:1-9.
22. Irwin M, Artin KH, Oxman MN. Screening for depression in the older adult: criterion validity of the 10-item Center for Epidemiological Studies Depression Scale (CES-D). Arch Intern Med. 1999;159(15):1701-4.
23. Woloshin S, Schwartz LM. Distribution of C-reactive protein values in the United States. N Engl J Med. 2005;352(15):1611-3.