Letter to the Editor

Circulating IL-32 and IL-33 levels in patients with asthma and COPD: a retrospective cross-sectional study

Min-Hye Kim[1], Jae-Woo Kwon[2]^, Jang-Hee Hahn[3], Minseo Kim[3], Hun Soo Chang[4], Jong Sook Park[5], Sang-Heon Cho[6], Choon-Sik Park[5]

[1]Department of Internal Medicine, Ewha Womans University College of Medicine, Seoul, Korea; [2]Department of Internal Medicine, Kangwon National University School of Medicine, Chuncheon, Korea; [3]Department of Anatomy and Cell Biology, Kangwon National University School of Medicine, Chuncheon, Korea; [4]Department of Anatomy and BK21 FOUR Project, College of Medicine, Soonchunhyang University, Cheonan, Korea; [5]Division of Allergy and Respiratory Medicine, Soonchunhyang University Bucheon Hospital, Bucheon, Korea; [6]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea

Correspondence to: Jong Sook Park, MD, PhD. Division of Allergy and Respiratory Medicine, Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, 1174, Jung-Dong, Wonmi-Ku, Bucheon, Kyeonggi-Do 420-020, South Korea. Email: js1221@schmc.ac.kr; Jae-Woo Kwon, MD, PhD. Department of Internal Medicine, Baengnyeong-ro 156, Chuncheon-Si, Gangwon-Do Kangwon National University Hospital, Chuncheon, Korea. Email: legent@hanmail.net.

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Interleukin-32 (IL-32) and IL-33 are innate cytokines that respond to various external stimuli and show diverse activities depending on the situation (1,2). In inflammatory airway diseases, IL-32 and IL-33 expressed in lung tissues show distinct features between chronic obstructive pulmonary disease (COPD) and asthma. IL-32 expression in the lungs of COPD patients is associated with neutrophilic inflammation and disease severity (3). IL-33 can induce eosinophilic inflammation, and increased IL-33 expression has been observed in the airways of asthma patients (1,4). We hypothesized that plasma IL-32 and IL-33 may be useful for distinguishing between asthma and COPD, and compared the plasma levels of IL-32γ, the most active isoform of IL-32 transcripts, and IL-33 in patients with asthma and COPD.

IL-32γ and IL-33 levels in the plasma of asthma patients (n=103), COPD patients (n=40), and healthy controls (n=51) were evaluated. Asthma was diagnosed by clinicians based on clinical symptoms. The diagnosis was supported by positive investigations for reversible airway obstruction (Appendix 1). Patients with asthma who were current smokers or ex-smokers with >10 pack-years were excluded. COPD was diagnosed by a pulmonologist on the basis of typical symptoms, a smoking history of >10 pack-years, and a pre-bronchodilator forced expiratory volume in 1 second (FEV_1) to forced vital capacity ratio <70%. The Biobank database was analyzed, but data on the post-bronchodilator lung function of COPD patients were not available. The characteristics of the study population are presented in Table S1.

The detection rates of plasma IL-33 were higher in asthmatic patients (80%) than in patients with COPD (65%) and controls (49%). Plasma IL-32γ was detected in most participants in all groups. Plasma levels of IL-32γ and IL-33 were higher in asthma patients than in COPD patients and controls [median (interquartile range): 2.73 (1.42–4.47) *vs.* 1.61 (0.74–2.68) *vs.* 1.76 (0.39–3.15) ng/mL, P=0.001; *Figure 1A*; and 3.56 (0.89–4.47) *vs.* 1.61 (0.00–3.78) *vs.* 0.00 (0.00–2.19) ng/mL, P<0.0001; *Figure 1B*] (Figure S1). Similar results were observed for log transformation of the data. Plasma levels of IL-32γ differed between patients with asthma and COPD following adjustment for FEV_1 (%) (P=0.004). However, neither cytokine showed significant differences among the three groups after adjustment for sex, age, and smoking history. Plasma IL-32γ and IL-33 levels were not related to the inflammatory or clinical characteristics of either disease (Table S2), except for a

^ ORCID: 0000-0003-1639-3606.

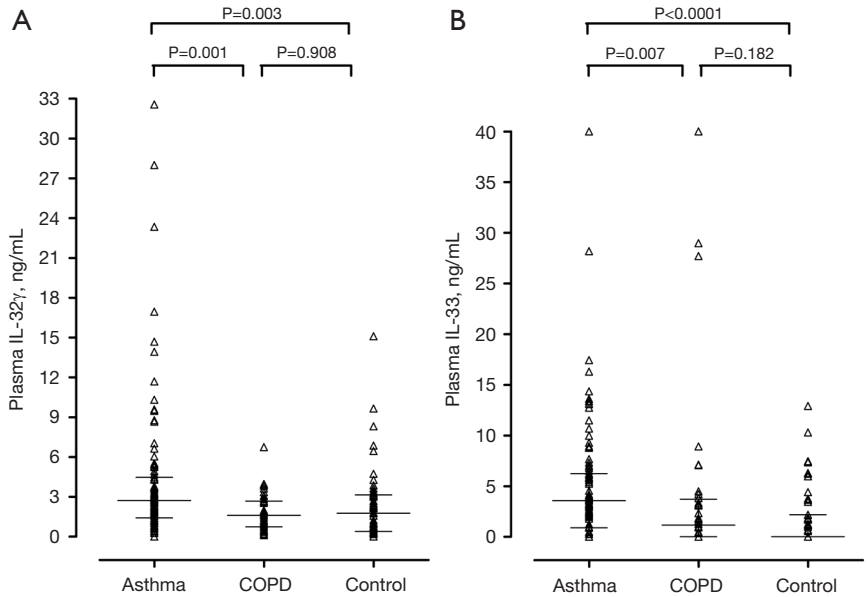

Figure 1 The levels of (A) plasma IL-32γ and (B) IL-33 in healthy controls, patients with asthma, and patients with COPD. The line represents median values with interquartile range. IL, interleukin; COPD, chronic obstructive pulmonary disease.

weak correlation between FEV_1 (%) and plasma IL-33 level in patients with asthma (r=–0.215, P=0.029), and a positive correlation between the plasma levels of IL-32γ and sputum eosinophilia in patients with COPD (r=0.358, P=0.032). There was a negative correlation between plasma IL-32γ levels and age among asthmatic patients, which requires further evaluation in larger studies. It was reported that serum levels of IL-32α and β were higher in asthma patients compared to controls, and that there were no differences in age among asthmatic patients according to the presence of serum IL-32 (5). And, Bang *et al.* (6) reported lower serum levels of IL-32γ in older asthmatic patients compared to healthy controls.

There were positive correlations between plasma IL-32γ and IL-33 in patients with asthma, patients with COPD, and healthy controls (r=0.720, P<0.0001; r=0.512, P=0.001; and r=0.784, P<0.0001, respectively) (Figure S2). This was unexpected and contrasted with previous reports of the roles of IL-32 and IL-33 in the lungs of asthma and COPD patients. Notably, no studies have evaluated IL-32 and IL-33 together. Immunohistochemical analyses showed that IL-32γ and IL-33 were co-expressed mainly in monocytes and the expression levels of both cytokines increased with the stimulation of GM-CSF and TNF-α (Figure S3).

The results regarding the relationships between the plasma levels of these cytokines and the inflammatory or clinical characteristics of patients have been inconsistent, which contrasts with the consistent results regarding the presence of these cytokines in asthma and COPD lung tissues. In contrast to the relationship between IL-32 expression and neutrophilic inflammation in the lung tissues of COPD patients, plasma IL-32γ levels in COPD patients were positively correlated with sputum eosinophilia in this study. As one of the diverse features of IL-32, IL-32γ could increase eosinophilic inflammation in response to infectious stimuli (7). The study by Meyer *et al.* (5) showed that serum IL-32 levels in asthma patients correlated with the responsiveness to asthma therapy, as reflected in improved FEV_1 and reduced blood eosinophil counts. Serum IL-32 levels are higher in patients with acute COPD exacerbations than asthmatic patients (8). However, several studies have reported that plasma IL-32 expression is not elevated in stable COPD patients (9,10). As innate cytokines and alarmins, plasma IL-32 and IL-33 are induced by various stimuli such as infections, cigarette smoke, and pollution, which could serve as common triggers for asthma and COPD exacerbations. Therefore, the plasma expression levels of IL-33 and IL-32 in asthma and COPD patients may be related to inflammatory activity. This is a well-established concept in other inflammatory diseases, such as rheumatoid arthritis and tuberculosis, but has not yet been applied to asthma and COPD.

The levels of both plasma IL-32γ and IL-33 were elevated in asthma patients, and the levels of these cytokines in plasma do not seem to reflect the differences in the

pathophysiologies of asthma and COPD. The levels of these innate cytokines in plasma were strongly positively correlated, and were co-expressed in monocytes in peripheral blood mononuclear cells. Plasma IL-32γ and IL-33 levels may be related to inflammatory activity in chronic airway inflammatory diseases.

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Footnote

Provenance and Peer Review: This article was a standard submission to the journal. The article has undergone external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-21-524/coif). All authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Methods

Study participants

Patients were enrolled from Soonchunhyang University Bucheon Hospital (11). Asthma was diagnosed by physicians based on the Global Initiative for Asthma guidelines (12). The diagnosis was supported by one or more of the following criteria: an increase in forced expiratory volume in one second (FEV_1) of >15%, or FEV_1 of 200 mL and 12% after 400 μg albuterol inhalation; a positive methacholine bronchial provocation test (MBPT) result, which was as defined as a concentration of methacholine that caused a 20% decrease in FEV_1 (PC_{20}) ≤10 mg per ml; or more than 20% increase in FEV_1 after 2-week treatment with inhaled or systemic corticosteroids. Asthmatic patients who were current smokers or ex-smokers with more than 10 pack-years were excluded. All included patients with COPD had pre-bronchodilator forced expiratory volume in 1 s/forced vital capacity (FEV_1/FVC) values of <0.7 and a smoking history of >10 pack-years. Control participants were selected using the criterion of normal spirometric values.

All enrolled patients were in stable condition when serum and sputum samples were collected. Patients with COPD or asthma exacerbations were excluded. Demographic characteristics including smoking status, results of induced-sputum analyses, pre-bronchodilator lung function tests, and eosinophil/neutrophil counts in peripheral blood were collected for the study populations. Serum levels of total IgE and results of induced sputum examination were collected for control participants. For patients with asthma, the following information was collected: onset age of asthma, asthma duration, post-bronchodilator spirometry, results of the methacholine provocation test PC_{20}, and number of subsequent exacerbations. Asthma exacerbation was defined as a short-term treatment with a systemic corticosteroid for increased typical asthmatic symptoms with FEV_1 < 80% of the personal best (12,13).

Informed written consent was obtained from all participants. All samples were obtained and processed according to the protocol of the Soonchunhyang University Bucheon Hospital biobank. Induced Sputum and specimen processing were performed as previously described protocols (14). The study was approved by the ethics committee of Kangwon National University Hospital (KNUH-2015-02-004-001) and Soonchunhyang University Bucheon Hospital (SCHGM 2014-16).

Quantitative measurement of cytokines

A sandwich ELISA kit (YbdY Biotechnology, Seoul, Korea) was used to measure IL-32γ (15) and IL-33 in duplicate according to the manufacturer's instructions with the lower limit of detection 0.082 ng/mL. The values less than the lower limit of detection were regarded as 0 ng/mL in statistical analyses. Cytokines were measured in duplicate and presented as mean values.

Immunocytochemistry

Peripheral blood mononuclear cells (PBMCs) were extracted from the buffy coat using Ficoll-Paque™ PREMIUM (17-5442-02, GE Healthcare, Chicago, IL, USA). To examine the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) or tumor necrosis factor alpha (TNF-α) on the induction of IL-32 or IL-33 expression, PBMCs were cultured in 24-well culture plates with or without TNF-α (ab9642, Abcam, Cambridge, MA, USA) and GM-CSF (sc-4585, Santa Cruz Biotechnology Inc., Dallas, TX, USA) for 24 hours. PBMCs were seeded in RPMI-1640 medium containing 10% fetal bovine serum, streptomycin, and penicillin with 1×10^6 cells/mL density. For immunocytochemical analyses, cells were fixed in 4% formaldehyde. The cell were washed with phosphate-buffered saline before permeabilization with 0.1% Triton X-100 (Sigma-Aldrich Inc., St. Louis, MO, USA), and then probed using a mouse monoclonal antibody to IL-32 (sc-517408, Santa Cruz Biotechnology Inc.) or IL-33 (ab207737, Abcam) for 60 min at room temperature. After repeated washes with phosphate-buffered saline, the cells were then probed with a secondary polyclonal antibody to mouse IgG1 conjugated to Alexa-488 (A32723, Thermo Fisher Scientific, Waltham, MA, USA) and rabbit IgG1 conjugated to rhodamine (R-6394, Thermo Fisher Scientific) for 40 min. The cells were washed again with phosphate-buffered saline and mounted on microscope slides with a mounting medium containing DAPI (DUO82040, Sigma-Aldrich Inc.) for nuclear staining. An Olympus FluoView FV1000 confocal microscope (Olympus, Tokyo, Japan) was used to obtain fluorescence images.

Statistical analyses

Continuous variables were presented as means and standard

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deviations (SD) and relative frequencies are presented for categorical variables in baseline characteristics. The normalities in data distributions were evaluated via the Shapiro–Wilk test, and the body mass index (BMI), FVC, and FEV_1 followed normal distributions. Plasma levels of cytokines, cell counts of induced sputum, and other variables which did not follow a normal distribution were analyzed with Spearman's rank correlation coefficient analysis. Kruskal–Wallis test and analysis of variance were used for comparisons in nonparametric and parametric variables, and then Mann–Whitney U tests or t-test were performed for post hoc analyses. Differences in plasma IL-32γ and IL-33 levels were compared among patients with asthma, patients with COPD, and healthy controls after adjustment for age and FEV_1 (%) using a general linear model. Statistical significance was accepted for P-values <0.05. All statistical analyses were performed using IBM SPSS Statistics, ver. 20.0 (IBM Corp., Armonk, NY, USA).

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Table S1 Characteristics of the study population

Characteristics	Asthma (n=103)	COPD (n=40)	Controls (n=51)	P value
Sex (M/F)	29/74	39/1	39/12	<0.0001
Age (range) in years	50.03 (17–80)	65.51 (47–84)	64.60 (40–86)	<0.0001
Onset age in years	42.80±16.19	–	–	
Duration of asthma in years	7.24±9.30	–	–	
Smoking History (NS/ES/SM)	85/13/5	0/22/18	17/10/24	<0.0001
Smoking amount (pack-year)	3.81±10.71	36.54±17.85	19.90±23.39	<0.0001
FVC, %	71.01±16.88	62.73±15.11	99.24±12.91	<0.0001
FEV_1, %	64.68±22.03	44.90±16.49	111.59±13.75	<0.0001
FEV_1/FVC	67.80±12.54	47.58±10.67	81.20±5.62	<0.0001
BMI	23.35±3.24	21.61±2.53	23.47±4.34	0.014
Bronchodilator FEV_1, %	12.94±11.51	–	–	
Bronchodilator FEV_1, L	0.19±0.18	–	–	
PC_{20}	6.33±9.16	–	–	
Total IgE	442.83±859.72	–	150.70±209.28	0.004
Exacerbation	65 (63.11%)	–	–	
Exacerbation (Y/N/ND)	65/18/11	–	–	
Sputum neutrophils %	62.28±32.89	84.77±17.20	53.99±37.10	0.003
Sputum eosinophils %	27.36±32.53	3.14±12.07	0.22±0.58	0.006
Detection rates of plasma IL-32γ	98 (95.1%)	40 (100%)	48 (94.1%)	0.323
Detection rates of plasma IL-33	82 (79.6%)	26 (65.0%)	25 (49.0%)	0.001

Mean±SD. FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; NS, non-smoker; ES, ex-smoker; SM, smoker; Y, yes; N, no; ND, not determined

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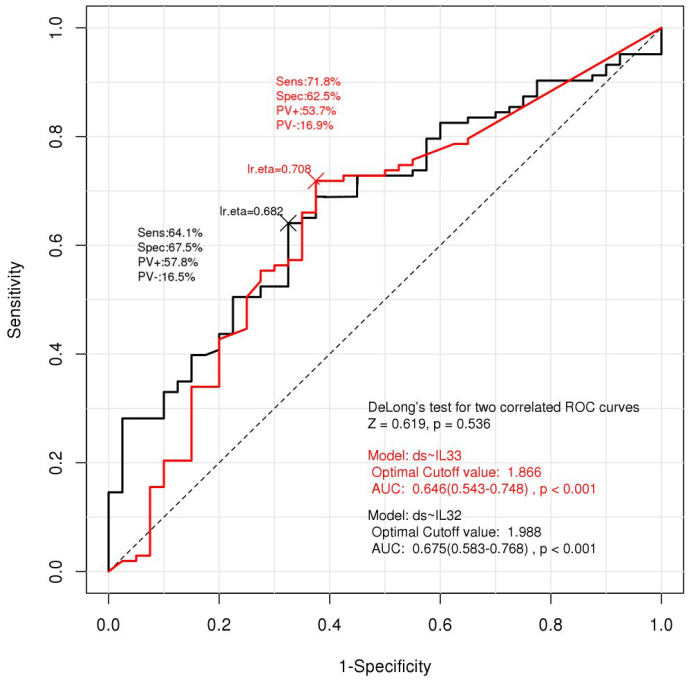
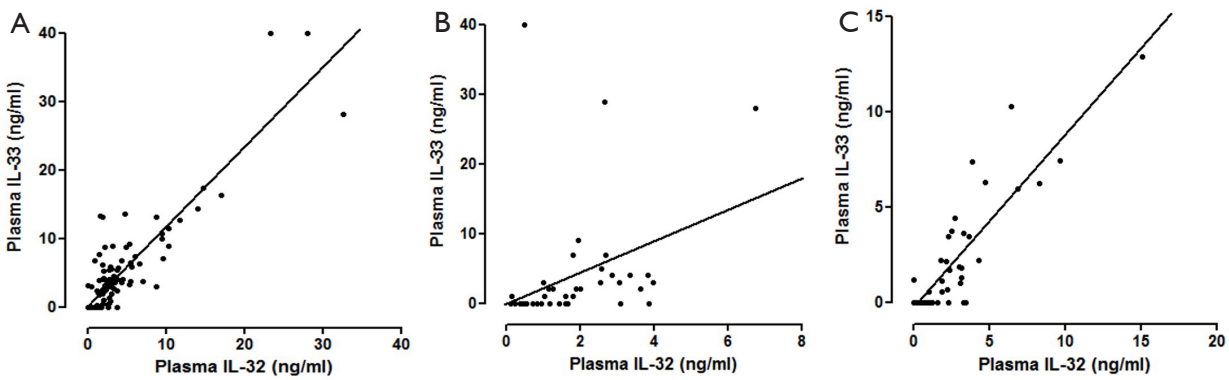


Figure S1 ROC curve to distinguish asthma from COPD.



Patients with asthma (r = 0.720, p < 0.0001) Patients with COPD (r = 0.512, p = 0.001) Healthy controls (r = 0.784, p < 0.0001)

Figure S2 Correlation between the levels of plasma IL-32γ and IL-33. (A) Asthma patients, (B) COPD patients, and (C) Healthy controls.

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Table S2 Characteristics of the study population

			Age	BMI	FVC%	FEV1%	FEV1/FVC	Sputum Neutrophili (%)	Sputum Eosinophil (%)	Total_IgE	PC20
Asthma	hIL-32 (ng/mL)	Correlation Coefficient	–0.395**	–0.230*	-0.061	-0.086	0.095	-0.133	0.122	0.019	-0.086
		P value	0.000	0.020	0.537	0.388	0.340	0.347	0.387	0.850	0.450
		Number	103	103	103	103	103	52	52	99	80
	hIL-33 (ng/mL)	Correlation Coefficient	–0.336**	–0.257**	–0.157	–0.215*	–0.017	–0.079	–0.022	0.039	-0.183
		P value	0.001	0.009	0.112	0.029	0.862	0.578	0.880	0.701	0.105
		Number	103	103	103	103	103	52	52	99	80
COPD	hIL-32 (ng/mL)	Correlation Coefficient	–0.038	–0.147	0.011	0.022	0.080	–0.269	0.358*		
		P value	0.816	0.365	0.945	0.894	0.623	0.112	0.032		
		Number	40	40	40	40	40	36	36		
	hIL-33 (ng/mL)	Correlation Coefficient	0.264	-0.150	0.149	0.220	.385*	–0.219	0.122		
		P value	0.100	0.354	0.358	0.172	0.014	0.199	0.478		
		Number	40	40	40	40	40	36	36		
Control	hIL-32 (ng/mL)	Correlation Coefficient	–0.042	–0.108	–0.221	–0.180	0.211			0.109	
		P value	0.770	0.452	0.120	0.205	0.137			0.467	
		Number	51	51	51	51	51			47	
	hIL-33 (ng/mL)	Correlation Coefficient	–0.270	–0.179	–0.204	–0.220	0.210			0.058	
		P value	0.056	0.208	0.150	0.121	0.140			0.696	
		Number	51	51	51	51	51			47	
All	hIL-32 (ng/mL)	Correlation Coefficient	-0.354**	-0.130	-0.128	-0.102	0.075	–0.251*	.250*	0.076	
		P value	0.000	0.071	0.076	0.157	0.301	0.019	0.019	0.362	
		Number	194	194	194	194	194	88	88	146	
	hIL-33 (ng/mL)	Correlation Coefficient	-0.356**	-0.190**	–0.218**	–0.212**	-0.014	-0.182	0.100	0.104	
		P value	0.000	0.008	0.002	0.003	0.851	0.089	0.353	0.213	
		Number	194	194	194	194	194	88	88	146	

*P<0.05, **P<0.01. BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second.

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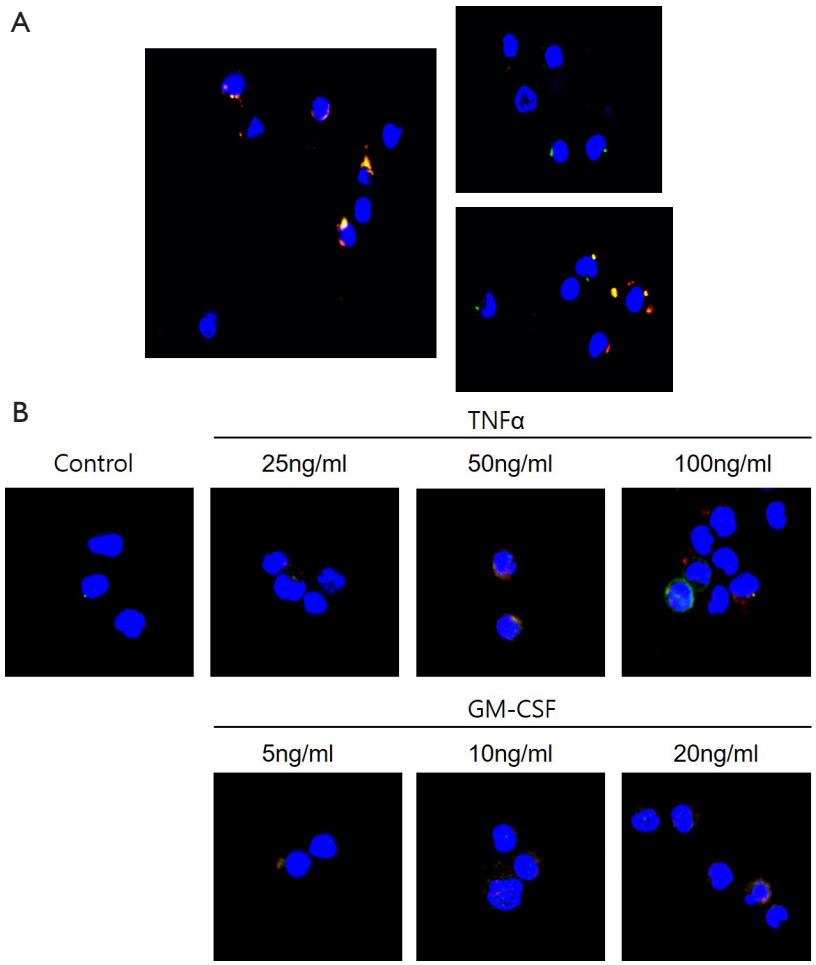

Figure S3 Immunofluorescence assay for peripheral blood mononuclear cells. (A) IL-32γ and IL-33 were expressed in monocytes. (B) Expression levels of IL-32γ and IL-33 were upregulated in the cytoplasm of monocytes among PBMCs stimulated with TNF-α and GM-CSF for 24 hours. IL-32: Alexa 488 (green), IL-33: rhodamine (red).

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