



Cigarette Smoke Extract Enhances IL-17A-Induced IL-8 Production via Up-Regulation of IL-17R in Human Bronchial Epithelial Cells

Kyoung-Hee Lee^{1,3}, Chang-Hoon Lee^{1,2,3}, Jisu Woo¹, Jiyeong Jeong¹, An-Hee Jang¹, and Chul-Gyu Yoo^{1,2,*}

¹Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Seoul National University Hospital, Seoul 03080, Korea, ²Department of Internal Medicine, Seoul National University College of Medicine, Seoul 03080, Korea, ³These authors contributed equally to this work.

*Correspondence: cgyoo@snu.ac.kr

<http://dx.doi.org/10.14348/molcells.2018.2123>

www.molcells.org

Interleukin-17A (IL-17A) is a pro-inflammatory cytokine mainly derived from T helper 17 cells and is known to be involved in the pathogenesis of chronic obstructive pulmonary disease (COPD). Cigarette smoke (CS) has been considered as a primary risk factor of COPD. However, the interaction between CS and IL-17A and the underlying molecular mechanisms have not been clarified. In the current study, we investigated the effects of cigarette smoke extract (CSE) on IL-17A-induced IL-8 production in human bronchial epithelial cells, and sought to identify the underlying molecular mechanisms. IL-8 production was significantly enhanced following treatment with both IL-17A and CSE, while treatment with either IL-17A or CSE alone caused only a slight increase in IL-8 production. CSE increased the transcription of *IL-17RA/RC* and surface membrane expression of IL-17R, which was suppressed by an inhibitor of the phosphoinositide 3-kinase (PI3K)/Akt pathway (LY294002). CSE caused inactivation of glycogen synthase kinase-3 β (GSK-3 β) via the PI3K/Akt pathway. Blockade of GSK-3 β inactivation by overexpression of constitutively active GSK-3 β (S9A) completely suppressed the CSE-induced up-regulation of IL-17R expression and the CSE-induced enhancement of IL-8 secretion. In conclusion, inactivation of GSK-3 β via the PI3K/Akt pathway mediates CSE-induced up-regulation of IL-17R, which contributes to the enhancement of IL-17A-induced IL-8 production.

Keywords: Akt, cigarette smoke extract, GSK-3 β , IL-17A, IL-17R, IL-8

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a significant cause of morbidity and mortality worldwide (Mannino and Buist, 2007). It is characterized by persistent and usually progressive airflow limitation, and is associated with a chronic inflammatory response in the airways to noxious particles or gases such as cigarette smoke (CS). Small airway inflammation and emphysema are the main pathologies of COPD (GOLD, 2016). An increase in the number of inflammatory cells, including cytotoxic T lymphocytes, macrophages and neutrophils, have been observed in patients with COPD, and these cells release inflammatory mediators and enzymes that interact with structural cells in the airways, parenchyma and vasculature (Hogg et al., 2004).

Interleukin-17 (IL-17) is a pro-inflammatory cytokine (Yao et al., 1995) mainly derived from T helper 17 (T_H17) cells (Harrington et al., 2005), which contributes to the pathogenesis of inflammatory disease (Fossiez et al., 1996). The IL-17 family consists of 6 members, IL-17A to F (Kolls and Linden, 2004), of which IL-17A is the most active inducer of

Received 12 July, 2017; revised 6 December, 2017; accepted 10 January, 2018; published online 21 February, 2018

eISSN: 0219-1032

© The Korean Society for Molecular and Cellular Biology. All rights reserved.

© This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/3.0/>.

inflammation (Zrioual et al., 2009), and five receptors, IL-17RA to IL-17RE (Aggarwal and Gurney, 2002). IL-17A activates pro-inflammatory signaling pathways through binding to the receptors IL-17RA and IL-17RC (Hot and Miossec, 2011). Fibroblasts, endothelial cells and epithelial cells are the major targets of IL-17A (Miossec and Kolls, 2012). A number of studies have demonstrated the contribution of IL-17 to the pathogenesis of COPD (Chen et al., 2011; Di Stefano et al., 2009; Doe et al., 2010; Shan et al., 2012).

Although the pathogenesis of COPD is complicated, inflammation, oxidative stress, and protease-antiprotease imbalance are regarded as a pathogenic triad in COPD (Fischer et al., 2011), and many studies have demonstrated cross talk among them (Gupta et al., 2016; Lee et al., 2015; Kirkham and Barnes, 2013; Rahman and Adcock, 2006; Ungurs et al., 2014). Cigarette smoke itself contains a high concentration of reactive oxygen species (ROS), and therefore there could be a relationship between cigarette smoke and IL-17-induced inflammation. However, this has not been investigated, and the underlying molecular mechanisms are unknown.

In the current study, we show that cigarette smoke extract (CSE) enhances IL-17A-induced IL-8 production via IL-17R up-regulation, and that this is mediated by activation of PI3K-Akt and subsequent inactivation of GSK-3 β .

MATERIALS AND METHODS

Cells and reagents

Normal human bronchial epithelial cells (BEAS-2B) were maintained in defined keratinocyte-SFM (Gibco by Life Technologies, USA) at 37°C under 5% CO₂. Recombinant human IL-17A (317-ILB-050), mouse monoclonal anti-human IL-17R-allophycocyanin (APC)-conjugated antibody, and an isotype control antibody were purchased from R&D Systems (USA). IL-17A was reconstituted to 100 μ g/ml in 4 mM HCl. A rabbit polyclonal anti-IL-17R antibody, to detect the N-terminal extracellular domain of IL-17R, anti-hemagglutinin (HA) and goat polyclonal anti-GAPDH antibodies were obtained from Santa Cruz Biotechnology (USA). Rabbit polyclonal anti-phospho-Akt (Ser473) (p-Akt), anti-phosphoglycogen synthase kinase-3 β (Ser9) (p-GSK-3 β), anti-phospho-p44/42 MAPK (Thr202/Tyr204) (p-ERK), and rabbit monoclonal anti-phospho-p38 MAPK (Thr180/Tyr182) (p-p38) antibodies were obtained from Cell Signaling (USA). U0126 (a highly selective inhibitor of MEK1 and MEK2), LY294002 (an inhibitor of phosphatidylinositol 3-kinase, PI3K), and SB203580 (a selective inhibitor of p38 MAPK) were obtained from Cell Signaling.

Cigarette smoke extract (CSE)

CSE was prepared as previously described (Lee et al., 2015). Briefly, commercial cigarettes (THIS; 84 mm long, 8 mm diameter; purchased from KT&G, Republic of Korea) were smoked continuously by a bottle system connected to a vacuum machine. Smoke from 20 cigarettes was bubbled into 60 mL phosphate-buffered saline (PBS) (Gibco). Large, insoluble particles in the resulting suspension were removed by filtering the solution through a 0.22- μ m filter.

Multiplex bead assay

IL-8 levels in culture supernatants were determined using a commercially available Bio-Plex Pro™ Cytokine Assay Kit (Bio-Rad, USA) according to the manufacturer's instructions.

Quantitative real-time PCR

Total RNA was isolated using an RNeasy Kit (Qiagen, Germany). cDNA was synthesized from 1 μ g of total RNA, using the Reverse Transcription System (Promega, USA). Power SYBR Green (Applied Biosystems, USA) was used for PCR amplification for IL-17RA, IL-17RC and GAPDH. Real-time PCR was performed using the following primers: human IL-17RA (fwd 5'-AGACTCCAGAACCAATTCC-3', rev 5'-TCTTAGAGTTGCTCTCCACCA-3'), human IL-17RC (fwd 5'-ACCAGAACCTCTGGCAAGC-3', rev 5'-GAGCTGTTACCTG AACACA-3') and GAPDH (fwd 5'-GAAGGTGAAGGTCG GAGTC-3', rev 5'-GAAGATGGTGATGGGATTTC-3').

Protein extraction and western blot analysis

Total cellular extracts were prepared in 1X cell lysis buffer (Cell Signaling). Membrane proteins were isolated using a Membrane Protein Extraction Kit (Thermo Fisher Scientific, USA). Equal amounts of protein were resolved by gradient SDS-polyacrylamide gel electrophoresis (Invitrogen) and transferred to nitrocellulose membranes (GE Healthcare, UK). Membranes were blocked with 5% skim milk blocking buffer for 1 h before being incubated overnight at 4°C with primary antibodies. Membranes were then washed with washing buffer three times and incubated with horseradish peroxidase-conjugated secondary antibodies in blocking buffer for 1 h. After successive washes, membranes were developed using a SuperSignal West Pico Chemiluminescent Kit (Thermo Fisher Scientific).

FACS analysis of the surface membrane receptor, IL-17R

Cells were washed twice in an isotonic PBS buffer supplemented with 0.5% BSA, then resuspended in the same buffer. Cells (1×10^5) were incubated with an APC-conjugated IL-17R antibody or an isotype control antibody for 45 min at 4°C. To remove unreacted IL-17R reagent, cells were washed twice in resuspension buffer. Fluorescence of individual cells was measured using a FACScan flow cytometer (BD, USA).

Transfection of plasmid vectors

A plasmid vector expressing GSK-3 β (S9A) (Ser9 to alanine substitution) and a control vector were used at a final concentration of 1 μ g. Transfection was performed using a Neon Transfection System (Thermo Fisher Scientific) according to the manufacturer's specifications. After 48 h, cells were used in experiments.

Statistical analysis

Data were subjected to Student's *t* test to determine statistical significance, and a P value of < 0.05 was considered significant.

RESULTS

CSE enhances IL-17A-induced IL-8 production

We first assessed the effect of CSE on IL-17A-induced IL-8

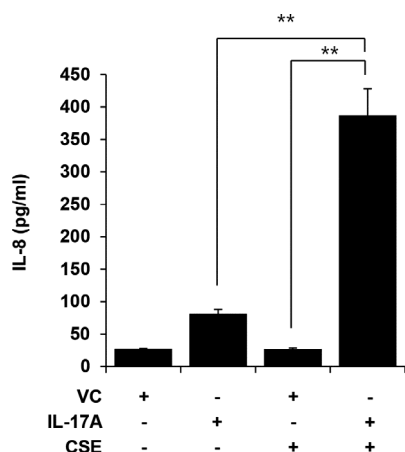


Fig. 1. CSE enhances IL-17A-induced IL-8 production. BEAS-2B cells were treated with CSE (1%) in the presence or absence of IL-17A (100 ng/ml) for 24 h. IL-8 concentration in cell culture media was measured using a multiplex bead assay. Data represent mean \pm SD of triplicates. $**P < 0.05$. Results are representative of three independent experiments.

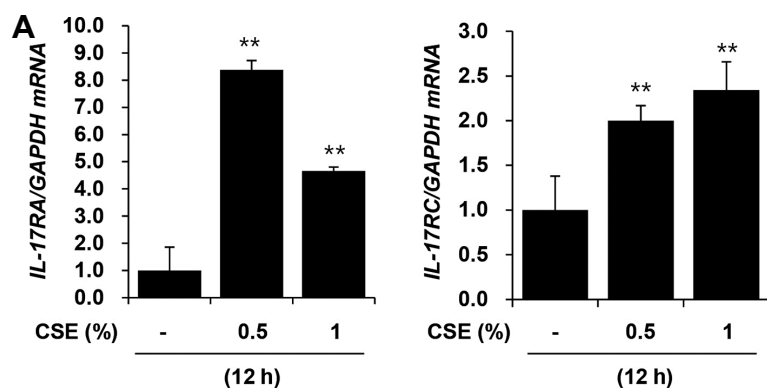
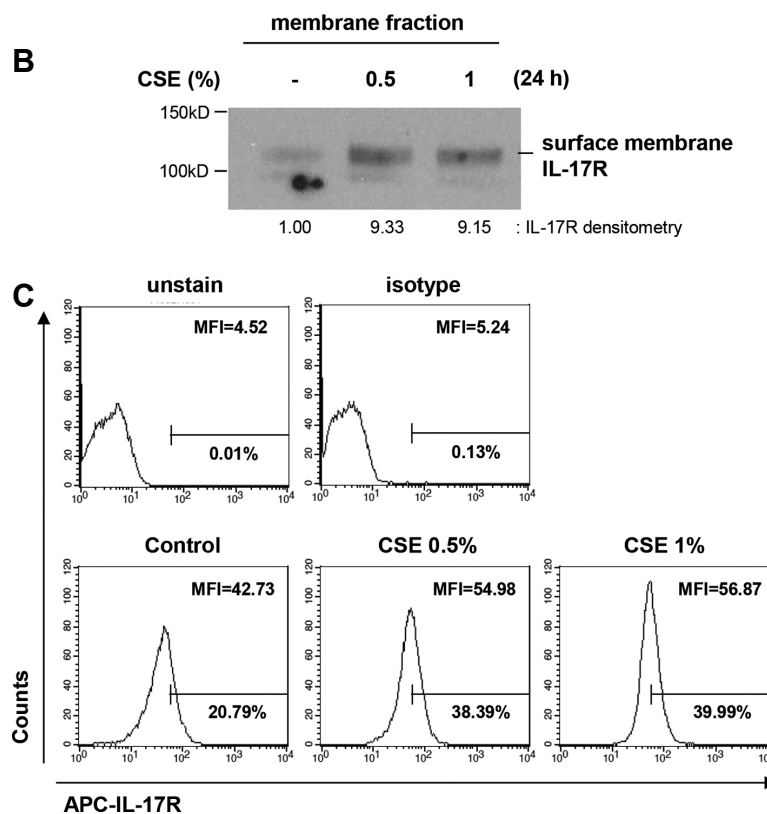


Fig. 2. CSE up-regulates IL-17R expression. BEAS-2B cells were treated with CSE (0.5, 1%) for 12 h (A) or 24 h (B, C). (A) Total RNA was isolated and quantitative real-time PCR for *IL-17RA*, *IL-17RC*, and *GAPDH* was performed. Data represent mean \pm SD of triplicates. $**P < 0.05$ versus control. (B) Membrane fraction was isolated and then subjected to western blot analysis for IL-17R to detect the N-terminal extracellular domain of IL-17RA/RC. (C) Cell surface expression of IL-17R was determined by flow cytometry. Results are shown as mean fluorescence intensity (MFI) histograms. Results are representative of three independent experiments.



production in BEAS-2B lung epithelial cells. While treatment with IL-17A alone led to a slight increase in IL-8 release, co-treatment with CSE significantly enhanced IL-17A-induced IL-8 production (Fig. 1).

CSE up-regulates IL-17RA/RC expression

IL-17A-induced production of IL-8 is known to be mediated by binding of IL-17A to its receptor, IL-17RA/RC. We therefore investigated whether CSE treatment influences the expression of IL-17RA/RC, and found that CSE increased IL-17RA and IL-17RC mRNA expression (Fig. 2A). To determine whether IL-17R expression was also up-regulated at the membrane level, we isolated membrane proteins from CSE-

treated cells and performed western blot analysis using an IL-17R antibody to detect the extracellular domain of IL-17R. Our results revealed that IL-17R in the membrane fraction was increased (Fig. 2B), and this was confirmed by flow cytometry (Fig. 2C). Taken together, these data suggest that CSE up-regulates IL-17R in lung epithelial cells.

Enhanced IL-17A-induced IL-8 production by CSE is dependent on PI3K/Akt activation

We next sought to determine the mechanism by which CSE up-regulates IL-17R expression. Previous reports have shown that CSE can activate both PI3K/Akt and mitogen-activated protein kinase (MAPK) signaling pathways (Kim et al., 2009;

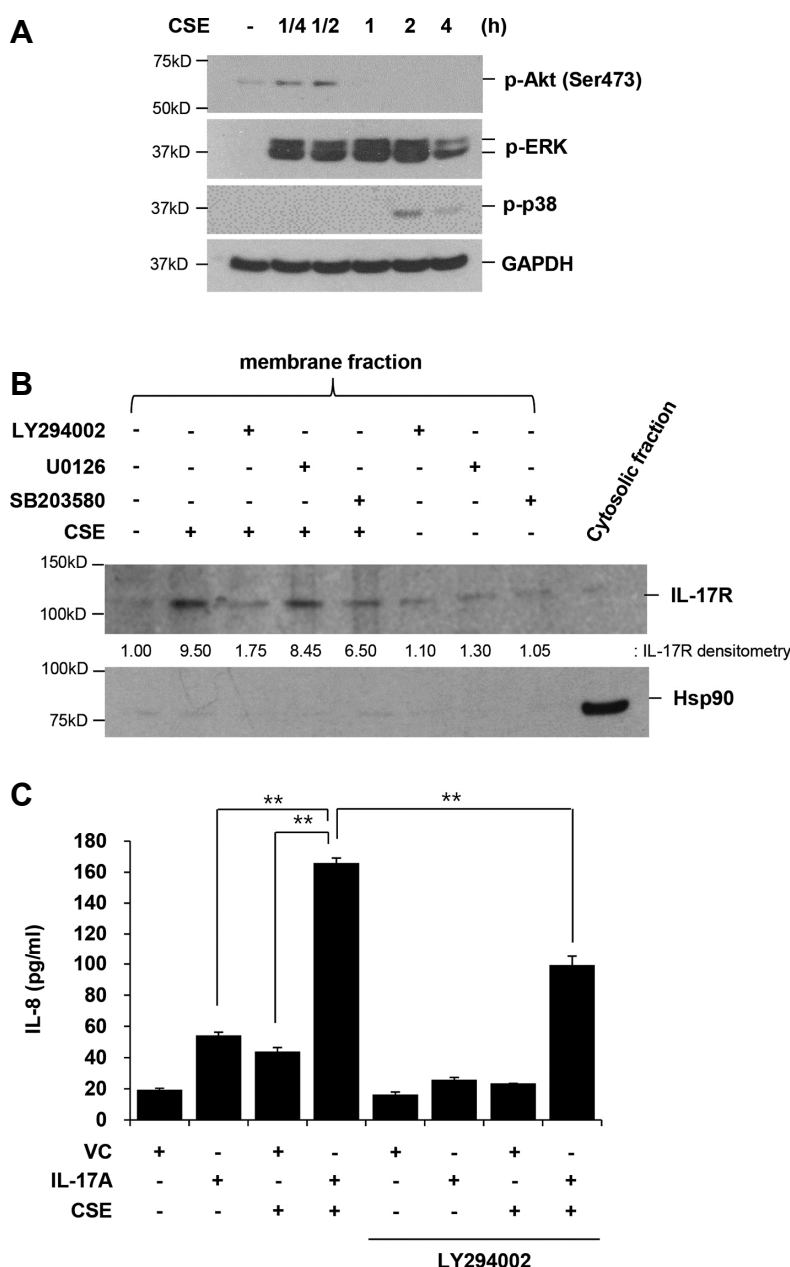


Fig. 3. Enhanced IL-17A-induced IL-8 production by CSE is dependent on PI3K/Akt activation.

(A) BEAS-2B cells were treated with CSE for the indicated times. Total cellular extracts were subjected to Western blot analysis for p-Akt (Ser473), p-ERK (Thr202/Tyr204), p-p38 (Thr180/Tyr182), and GAPDH. (B) Cells were pretreated with a MEK1/2 inhibitor (U0126, 2 μ M), PI3K/Akt inhibitor (LY294002, 10 μ M), or p38 inhibitor (SB203580, 10 μ M) for 2 h then stimulated with CSE for 24 h in the presence of U0126, LY294002, or SB203580. Membrane proteins were extracted and subjected to Western blot analysis for IL-17R. Cytosolic proteins were subjected to western blot analysis for heat shock protein 90 (Hsp90). (C) BEAS-2B cells were pretreated with LY294002 (10 μ M) for 1 h then stimulated with CSE and IL-17A in the presence or absence of LY294002 for 24 h. IL-8 concentration in culture media was determined using a multiplex bead assay. Data represent mean \pm SD of triplicates. **P < 0.05. Results are representative of three independent experiments.

Lee et al., 2015). Thus, we sought to investigate whether these pathways are involved in the CSE-induced up-regulation of IL-17R in our system. Our results showed that CSE treatment activated Akt and MAPK (ERK and p38) in lung epithelial cells (Fig. 3A). Furthermore, pretreatment with an inhibitor of the PI3K/Akt pathway (LY294002) significantly suppressed IL-17R expression in the membrane fraction of CSE-treated cells (Fig. 3B). In accordance with these findings, PI3K/Akt inhibition partially suppressed the CSE-induced enhancement of IL-8 production in IL-17A-treated cells (Fig. 3C). These results indicate that enhanced IL-17A-induced IL-8 production by CSE is due, at least in part, to the PI3K/Akt mediated up-regulation of IL-17R.

Increased IL-17R through PI3K/Akt activation is mediated by GSK-3 β inactivation

An increase in phosphorylated-Akt was first seen 15 min

after CSE treatment, and returned to basal levels after 1 h. GSK-3 β is a well-known downstream molecule of Akt. Thus, as Akt inactivates GSK-3 β , we investigated whether GSK-3 β has a role in the CSE-induced increase in IL-17R. GSK-3 β is known to be constitutively active and is inactivated by phosphorylation at serine 9 (Stambolic and Woodgett, 1994). In the present study, we showed that inactive phosphorylated GSK-3 β at serine 9 was increased by CSE in a time-dependent manner (Fig. 4A). When Akt activation was inhibited by LY294002 pretreatment, GSK-3 β inactivation by CSE was markedly suppressed (Fig. 4B). This suggests that CSE inactivates GSK-3 β via activation of the PI3K/Akt pathway. To further evaluate the role of GSK-3 β inactivation in CSE-induced IL-17R expression, we determined whether the effect of CSE was blocked by active GSK-3 β overexpression. Cells were transiently transfected with either GSK-3 β (S9A), a plasmid vector expressing HA-tagged GSK-3 β cDNA where

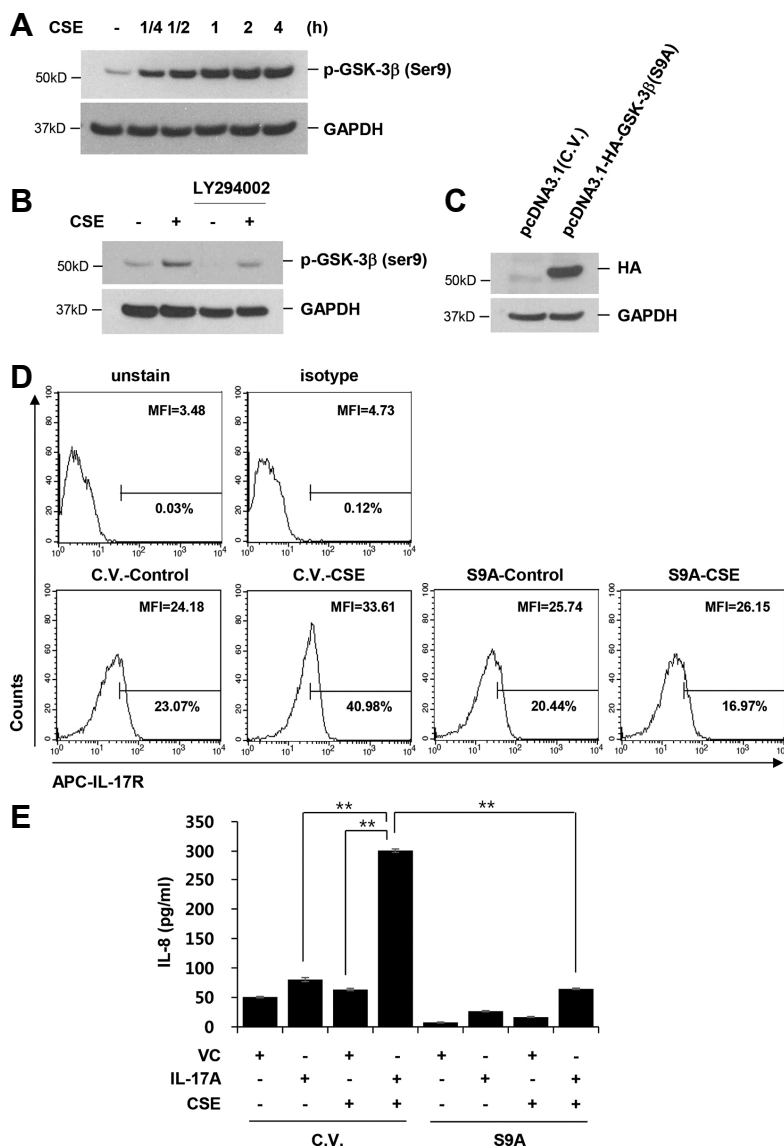


Fig. 4. Increased IL-17R through PI3K/Akt activation is mediated by GSK-3 β inactivation.

(A) BEAS-2B cells were treated with CSE for the indicated times. Expression of p-GSK-3 β (Ser9) was assessed by Western blot analysis. (B) Cells were pretreated with PI3K/Akt inhibitor (LY294002, 10 μ M) for 1 h then stimulated with CSE in the presence or absence of LY294002 for 1 h. Total cellular extracts were subjected to Western blot analysis for p-GSK-3 β (Ser9) and GAPDH. (C-E) Cells were transiently transfected with an HA-tagged GSK-3 β (S9A) expression vector or a control vector (C.V., pcDNA3.1). After 48 h, cells were treated with CSE for 24 h. Total cellular extracts were subjected to western blot analysis for HA and GAPDH (C). Cell surface expression of IL-17R was determined by flow cytometry (D). IL-8 concentration in culture media was determined using a multiplex bead assay (E). Data represent mean \pm SD of triplicates. **P < 0.05. Results are representative of three independent experiments.

serine 9 was substituted for non-phosphorylatable alanine, or a control vector (pcDNA3.1). Because GSK-3 β (S9A) cannot be inactivated, it functions as a constitutively active form of GSK-3 β . Overexpression of GSK-3 β (S9A) was confirmed using immunoblotting against HA (Fig. 4C). CSE-induced IL-17R expression and CSE-induced enhancement of IL-8 production were completely blocked in GSK-3 β (S9A)-overexpressing cells, compared to control vector-transfected cells (Figs. 4D and 4E). Taken together, these findings indicate that GSK-3 β inactivation via the PI3K/Akt pathway mediates CSE-induced up-regulation of IL-17R.

DISCUSSION

COPD is characterized by anomalous and persistent inflammation, both local and systemic. Neutrophilic inflammation predominates in the COPD airway wall and lumen. CS induces emphysema and increases lung IL-17A production in mice (Shan et al., 2012). The number of inflammatory cells including neutrophils is attenuated in bronchoalveolar lavage fluid of *Il-17a*^{-/-} mice when compared to that of wild-type mice. Smoke-induced emphysema is also attenuated in *Il-17a*^{-/-} mice. In contrast, overexpression of IL-17A in the airways of transgenic mice accelerates the development of emphysema and neutrophilic inflammation after CS exposure (Shan et al., 2012). These findings suggest that IL-17A and IL-17A-induced neutrophilic inflammation are important for COPD pathogenesis.

IL-17 is known to contribute to neutrophilic airway inflammation by the release of neutrophil-mobilizing cytokines from airway cells. IL-8 is the major neutrophil chemotactic factor in the lung (Kunkel et al., 1991). In the present study, and consistent with previous reports, we showed that IL-17A led to the production of IL-8 in human bronchial epithelial cells (Bettelli et al., 2007). Released IL-8 from lung epithelial cells recruits neutrophils into the lung and thus further amplifies chronic inflammation.

T_H17 cells, the major source of IL-17, produce more inflammatory cytokines than Tregs or other T-helper cells (Burgler et al., 2009). T_H17 cells (Vargas-Rojas et al., 2011) and IL-17-positive cells (Di Stefano et al., 2009; Doe et al., 2010) are reported to be increased in the peripheral blood and airways of patients with COPD. The sputum concentration of IL-8 is significantly higher in the patients with COPD than in the control subject (Yamamoto et al., 1997). Together, all of these findings indicate that IL-17A contributes to the pathogenesis of COPD and IL-17A-induced IL-8 production mediates at least partially the airway neutrophilic inflammation.

Moreover, the T_H17 lineage requires optimum concentrations of TGF- β and IL-6 in the surrounding microenvironment (Mangan et al., 2006), both of which are elevated in the serum and sputum of patients with COPD (Mak et al., 2009; Perera et al., 2007). IL-17 has also been linked to increased mucus production via its effects on *mucin* gene expression in the airway epithelium (Onishi and Gaffen, 2010). These results suggest that IL-17 could be a plausible treatment target for COPD (Cazzola and Matera, 2012).

A recent report showed that CS acts as a T_H17 cell adjuvant by enhancing T_H17 cell differentiation *in vitro* and *in*

in vivo (Chen et al., 2011). However, the existence of cross talk between CS and IL-17A has not been clarified. Our study showed that CSE enhanced IL-17A-induced IL-8 production via up-regulation of IL-17R, revealing that cross talk between CSE and IL-17A increases airway inflammation. Although CS is a well-known cause of COPD, and is widely used in murine models of COPD, we chose to use CSE instead of CS in the current study because we had previously developed a new emphysema model in mice using an intra-tracheal injection of CSE (Lee et al., 2015; 2017).

GSK-3 β , one of two isoforms of glycogen synthase kinase 3, is a Ser/Thr kinase (Doble and Woodgett, 2003) that regulates more than 50 substrates and many essential cellular functions, such as glycogen metabolism, cell cycle control, cell differentiation and inflammation (Cortes-Vieyra et al., 2012; Jope and Johnson, 2004). GSK-3 β is activated by Tyr216 phosphorylation and is inactivated by Ser9 phosphorylation. Several protein kinases can phosphorylate Ser9, including protein kinase A (PKA), protein kinase B (PKB, Akt), and ribosomal protein S6 kinase (S6K). Of these, the most important GSK-3 β inactivator is Akt (Cortes-Vieyra et al., 2012; Jope and Johnson, 2004), which is activated by PI3K (Mahajan and Mahajan, 2012). In the present study, CSE was shown to activate Akt and phosphorylate GSK-3 β (Ser9). CSE-induced up-regulation of IL-17R was completely inhibited by a constitutively active form of GSK-3 β (S9A), which was not inactivated by Akt. The CSE-induced up-regulation of IL-17R was also suppressed by a chemical inhibitor of PI3K/Akt (LY294002). Other studies have reported that GSK-3 β inhibition increases IL-8 production through protein kinase C (PKC)-extracellular signal-related kinase (ERK) signaling (Wang et al., 2006). However, in the current study, the inhibition of ERK did not affect IL-17R expression. GSK-3 has been reported to regulate the activity of numerous factors including c-Jun (Boyle et al., 1991), c-Myc (Gregory et al., 2003), β -catenin (Rubinfeld et al., 1996; Thomas et al., 1999), heat shock factor 1 (Chu et al., 1998), NF- κ B (Hoefflich et al., 2000), signal transducers and activators of transcription (STAT) (Ginger et al., 2000), and MAPK/ERK kinase kinase 1 (MEK1) (Kim et al., 2003). At this time, it is not clear whether the CSE-induced up-regulation of IL-17R is mediated directly by inactive GSK-3 β or via the regulation of other signaling molecules.

In conclusion, CSE enhanced the IL-17A-induced IL-8 production by up-regulation of IL-17R, which is caused by CSE-induced activation of the PI3K/Akt pathway and subsequent inactivation of GSK-3 β .

ACKNOWLEDGMENTS

This work was supported by the Seoul National University Hospital [grant numbers 0320140450].

REFERENCES

- Aggarwal, S., and Gurney, A.L. (2002). IL-17: prototype member of an emerging cytokine family. *J. Leukoc. Biol.* 71, 1-8.
- Bettelli, E., Korn, T., and Kuchroo, V.K. (2007). Th17: the third member of the effector T cell trilogy. *Curr. Opin. Immunol.* 19, 652-657.

- Boyle, W.J., Smeal, T., Defize, L.H., Angel, P., Woodgett, J.R., Karin, M., and Hunter, T. (1991). Activation of protein kinase C decreases phosphorylation of c-Jun at sites that negatively regulate its DNA-binding activity. *Cell* *64*, 573-584.
- Burgler, S., Ouaked, N., Bassin, C., Basinski, T.M., Mantel, P.Y., Siegmund, K., Meyer, N., Akdis, C.A., and Schmidt-Weber, C.B. (2009). Differentiation and functional analysis of human T(H)17 cells. *J. Allergy Clin. Immunol.* *123*, 588-595, 595 e581-587.
- Cazzola, M., and Matera, M.G. (2012). IL-17 in chronic obstructive pulmonary disease. *Expert. Rev. Respir. Med.* *6*, 135-138.
- Chen, K., Pociask, D.A., McAleer, J.P., Chan, Y.R., Alcorn, J.F., Kreindler, J.L., Keyser, M.R., Shapiro, S.D., Houghton, A.M., Kolls, J.K., et al. (2011). IL-17RA is required for CCL2 expression, macrophage recruitment, and emphysema in response to cigarette smoke. *PLoS One* *6*, e20333.
- Chu, B., Zhong, R., Soncin, F., Stevenson, M.A., and Calderwood, S.K. (1998). Transcriptional activity of heat shock factor 1 at 37 degrees C is repressed through phosphorylation on two distinct serine residues by glycogen synthase kinase 3 and protein kinases Calpha and Czeta. *J. Biol. Chem.* *273*, 18640-18646.
- Cortes-Vieyra, R., Bravo-Patino, A., Valdez-Alarcon, J.J., Juarez, M.C., Finlay, B.B., and Baizabal-Aguirre, V.M. (2012). Role of glycogen synthase kinase-3 beta in the inflammatory response caused by bacterial pathogens. *J. Inflamm. (Lond)* *9*, 23.
- Di Stefano, A., Caramori, G., Gnemmi, I., Contoli, M., Vicari, C., Capelli, A., Magno, F., D'Anna, S.E., Zanini, A., Brun, P., et al. (2009). T helper type 17-related cytokine expression is increased in the bronchial mucosa of stable chronic obstructive pulmonary disease patients. *Clin. Exp. Immunol.* *157*, 316-324.
- Doble, B.W., and Woodgett, J.R. (2003). GSK-3: tricks of the trade for a multi-tasking kinase. *J. Cell. Sci.* *116*, 1175-1186.
- Doe, C., Bafadhel, M., Siddiqui, S., Desai, D., Mistry, V., Rugman, P., McCormick, M., Woods, J., May, R., Sleeman, M.A., et al. (2010). Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest* *138*, 1140-1147.
- Fischer, B.M., Pavlisko, E., and Voynow, J.A. (2011). Pathogenic triad in COPD: oxidative stress, protease-antiprotease imbalance, and inflammation. *Int. J. Chron. Obstruct. Pulmon. Dis.* *6*, 413-421.
- Fossiez, F., Djossou, O., Chomarat, P., Flores-Romo, L., Ait-Yahia, S., Maat, C., Pin, J.J., Garrone, P., Garcia, E., Saeland, S., et al. (1996). T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J. Exp. Med.* *183*, 2593-2603.
- Ginger, R.S., Dalton, E.C., Ryves, W.J., Fukuzawa, M., Williams, J.G., and Harwood, A.J. (2000). Glycogen synthase kinase-3 enhances nuclear export of a Dictyostelium STAT protein. *EMBO J.* *19*, 5483-5491.
- GOLD (2016). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease.
- Gregory, M.A., Qi, Y., and Hann, S.R. (2003). Phosphorylation by glycogen synthase kinase-3 controls c-myc proteolysis and subnuclear localization. *J. Biol. Chem.* *278*, 51606-51612.
- Gupta, V., Khan, A., Higham, A., Lemon, J., Sriskantharajah, S., Amour, A., Hessel, E.M., Southworth, T., and Singh, D. (2016). The effect of phosphatidylinositol-3 kinase inhibition on matrix metalloproteinase-9 and reactive oxygen species release from chronic obstructive pulmonary disease neutrophils. *Int. Immunopharmacol.* *35*, 155-162.
- Harrington, L.E., Hatton, R.D., Mangan, P.R., Turner, H., Murphy, T.L., Murphy, K.M., and Weaver, C.T. (2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* *6*, 1123-1132.
- Hoeflich, K.P., Luo, J., Rubie, E.A., Tsao, M.S., Jin, O., and Woodgett, J.R. (2000). Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. *Nature* *406*, 86-90.
- Hogg, J.C., Chu, F., Utokaparch, S., Woods, R., Elliott, W.M., Buzatu, L., Cherniack, R.M., Rogers, R.M., Sciurba, F.C., Coxson, H.O., et al. (2004). The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N. Engl. J. Med.* *350*, 2645-2653.
- Hot, A., and Miossec, P. (2011). Effects of interleukin (IL)-17A and IL-17F in human rheumatoid arthritis synoviocytes. *Ann. Rheum. Dis.* *70*, 727-732.
- Jope, R.S., and Johnson, G.V. (2004). The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem. Sci.* *29*, 95-102.
- Kim, J.W., Lee, J.E., Kim, M.J., Cho, E.G., Cho, S.G., and Choi, E.J. (2003). Glycogen synthase kinase 3 beta is a natural activator of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1 (MEKK1). *J. Biol. Chem.* *278*, 13995-14001.
- Kim, S.E., Thanh Thuy, T.T., Lee, J.H., Ro, J.Y., Bae, Y.A., Kong, Y., Ahn, J.Y., Lee, D.S., Oh, Y.M., Lee, S.D., et al. (2009). Simvastatin inhibits induction of matrix metalloproteinase-9 in rat alveolar macrophages exposed to cigarette smoke extract. *Exp. Mol. Med.* *41*, 277-287.
- Kirkham, P.A., and Barnes, P.J. (2013). Oxidative stress in COPD. *Chest* *144*, 266-273.
- Kolls, J.K., and Linden, A. (2004). Interleukin-17 family members and inflammation. *Immunity* *21*, 467-476.
- Kunkel, S.L., Standiford, T., Kasahara, K., and Strieter, R.M. (1991). Interleukin-8 (IL-8): the major neutrophil chemotactic factor in the lung. *Exp. Lung Res.* *17*, 17-23.
- Lee, K.H., Lee, C.H., Jeong, J., Jang, A.H., and Yoo, C.G. (2015). Neutrophil elastase differentially regulates interleukin 8 (IL-8) and vascular endothelial growth factor (VEGF) production by cigarette smoke extract. *J. Biol. Chem.* *290*, 28438-28445.
- Lee, C.H., Lee, K.H., Jang, A.H., and Yoo, C.G. (2017). The Impact of Autophagy on the Cigarette Smoke Extract-Induced Apoptosis of Bronchial Epithelial Cells. *Tuberc. Respir. Dis. (Seoul)* *80*, 83-89.
- Mahajan, K., and Mahajan, N.P. (2012). PI3K-independent AKT activation in cancers: a treasure trove for novel therapeutics. *J. Cell. Physiol.* *227*, 3178-3184.
- Mak, J.C., Chan-Yeung, M.M., Ho, S.P., Chan, K.S., Choo, K., Yee, K.S., Chau, C.H., Cheung, A.H., Ip, M.S., and Members of Hong Kong Thoracic Society, COPD Study Group. (2009). Elevated plasma TGF-beta1 levels in patients with chronic obstructive pulmonary disease. *Respir. Med.* *103*, 1083-1089.
- Mangan, P.R., Harrington, L.E., O'Quinn, D.B., Helms, W.S., Bullard, D.C., Elson, C.O., Hatton, R.D., Wahl, S.M., Schoeb, T.R., and Weaver, C.T. (2006). Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* *441*, 231-234.
- Mannino, D.M., and Buist, A.S. (2007). Global burden of COPD: risk factors, prevalence, and future trends. *Lancet* *370*, 765-773.
- Miossec, P., and Kolls, J.K. (2012). Targeting IL-17 and TH17 cells in chronic inflammation. *Nat. Rev. Drug Discov.* *11*, 763-776.
- Onishi, R.M., and Gaffen, S.L. (2010). Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. *Immunology* *129*, 311-321.
- Perera, W.R., Hurst, J.R., Wilkinson, T.M., Sapsford, R.J., Mullerova, H., Donaldson, G.C., and Wedzicha, J.A. (2007). Inflammatory changes, recovery and recurrence at COPD exacerbation. *Eur. Respir. J.* *29*, 527-534.
- Rahman, I., and Adcock, I.M. (2006). Oxidative stress and redox regulation of lung inflammation in COPD. *Eur. Respir. J.* *28*, 219-242.

- Rubinfeld, B., Albert, I., Porfiri, E., Fiol, C., Munemitsu, S., and Polakis, P. (1996). Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. *Science* *272*, 1023-1026.
- Shan, M., Yuan, X., Song, L.Z., Roberts, L., Zarinkamar, N., Seryshev, A., Zhang, Y., Hilsenbeck, S., Chang, S.H., Dong, C., et al. (2012). Cigarette smoke induction of osteopontin (SPP1) mediates T(H)17 inflammation in human and experimental emphysema. *Sci. Transl. Med.* *4*, 117ra119.
- Stambolic, V., and Woodgett, J.R. (1994). Mitogen inactivation of glycogen synthase kinase-3 beta in intact cells via serine 9 phosphorylation. *Biochem. J.* *303 (Pt 3)*, 701-704.
- Thomas, G.M., Frame, S., Goedert, M., Nathke, I., Polakis, P., and Cohen, P. (1999). A GSK3-binding peptide from FRAT1 selectively inhibits the GSK3-catalysed phosphorylation of axin and beta-catenin. *FEBS Lett.* *458*, 247-251.
- Ungurs, M.J., Sinden, N.J., and Stockley, R.A. (2014). Progranulin is a substrate for neutrophil-elastase and proteinase-3 in the airway and its concentration correlates with mediators of airway inflammation in COPD. *Am. J. Physiol. Lung Cell. Mol. Physiol.* *306*, L80-87.
- Vargas-Rojas, M.I., Ramirez-Venegas, A., Limon-Camacho, L., Ochoa, L., Hernandez-Zenteno, R., and Sansores, R.H. (2011). Increase of Th17 cells in peripheral blood of patients with chronic obstructive pulmonary disease. *Respir. Med.* *105*, 1648-1654.
- Wang, Q., Zhou, Y., Wang, X., and Evers, B.M. (2006). Glycogen synthase kinase-3 is a negative regulator of extracellular signal-regulated kinase. *Oncogene* *25*, 43-50.
- Yamamoto, C., Yoneda, T., Yoshikawa, M., Fu, A., okuyama, T., Tsukaguchi, K., and Narita, N. (1997). Airway inflammation in COPD assessed by sputum levels of interleukin-8. *Chest* *112*, 505-510.
- Yao, Z., Painter, S.L., Fanslow, W.C., Ulrich, D., Macduff, B.M., Spriggs, M.K., and Armitage, R.J. (1995). Human IL-17: a novel cytokine derived from T cells. *J. Immunol.* *155*, 5483-5486.
- Zrioual, S., Ecochard, R., Tournadre, A., Lenief, V., Cazalis, M.A., and Miossec, P. (2009). Genome-wide comparison between IL-17A- and IL-17F-induced effects in human rheumatoid arthritis synoviocytes. *J. Immunol.* *182*, 3112-3120.