



The activation of NLRP3-inflammasome by stimulation of diesel exhaust particles in lung tissues from emphysema model and RAW 264.7 cell line

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Background/Aims: Diesel exhaust particles (DEPs) lead to elevation of reactive oxygen species, which can activate the nucleotide-binding oligomerization domain-like receptor (NLR) family members containing the pyrin domain 3 (NLRP3)-inflammasome. In this study, we elucidated whether NLRP3-inflammasome is activated by DEPs and whether antioxidants (N-acetylcysteine [NAC]) could inhibit such activation.

Methods: RAW 264.7 cells and *ex vivo* lung tissues explants obtained from elastase-induced emphysema animal models were stimulated with cigarette smoking extract (CSE), DEPs, and lipopolysaccharide, and levels of interleukin-1 β (IL-1 β), caspase-1 and nucleotide-binding oligomerization domain-like receptor (NLR) family members containing the pyrin domain (NLRP3)-inflammasome were assessed by Western blotting and immunohistochemistry.

Results: NAC and caspase-1 inhibitor suppressed CSE- and DEP-induced secretion of IL-1 β in RAW 264.7 cells. The expression levels of the NLRP3-inflammasome and caspase-1 were upregulated in RAW 264.7 cells by stimulation with CSE and DEPs and were inhibited by NAC. CSE and DEPs increased the secretion of IL-1 β in lung tissues from both the normal and elastase-induced emphysema groups. The secretion of IL-1 β by CSE and DEPs was increased in the elastin-induced emphysema group more than that in the normal group (CSE: 309 \pm 19 pg/mL vs. 151 \pm 13 pg/mL, respectively, $p < 0.05$; DEP: 350 \pm 24 pg/mL vs. 281 \pm 15 pg/mL, respectively, $p < 0.05$). NAC inhibited CSE- and DEP-induced IL-1 β secretion in both the normal and elastase-induced emphysema groups. NLRP3-inflammasome expression as determined by immunohistochemistry was increased by CSE and DEPs in both the normal and elastin-induced emphysema groups, and was suppressed by NAC.

Conclusions: The NLRP3-inflammasome is activated by DEPs in *ex vivo* tissue explants from elastase-induced emphysema animal model, and this activation is inhibited by NAC.

Keywords: Pulmonary disease, chronic obstructive; Emphysema; Inflammasomes; Vehicle emissions; Pancreatic elastase

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is defined as a common, preventable, and treatable disease characterized by limited airflow that is persistent, typically progressive, and associated with chronic pulmonary inflammatory responses to noxious particles and gases [1]. The incidence of COPD has increased in the past few decades and is estimated to be ranked as the third most common cause of death by 2020 [2]. A previous cross-sectional study reported that the mortality rate increases to 2.5% in COPD patients with acute exacerbation of COPD (AECOPD) [3]. In addition, acute exacerbation can reduce long-term survival of patients with COPD [4]. The two known common causes of AECOPD appear to be respiratory infections and air pollution.

The inflammasome consists of nucleotide-binding oligomerization domain-like receptor (NLR) family members containing the pyrin domain (NLRP), the adaptor molecule apoptosis-associated speck-like protein containing a CARD (ASC) domain, and caspase-1 [5]. In response to pathogens, sterile tissue damage, and metabolic stress, NLRP3 binds to procaspase-1 through ASC, subsequently activating caspase-1. Activation of caspase-1 leads to maturation of interleukin-1 β (IL-1 β) [5,6]. Recently, the NLRP3-inflammasome has been hypothesized to be involved in the pathogenesis of COPD, although no direct evidence has been reported. Cigarette smoking elevates IL-1 β levels in the human lung [7]. IL-18, a member of the IL-1 cytokine family, is also elevated in the sputum and serum of patients with COPD [8,9]. These findings suggest that inflammasome is closely related to the pathogenesis of COPD. Furthermore, AECOPD may be related to NLRP3-inflammasome because about two-thirds of AECOPD cases are associated with viral or bacterial infection of the respiratory tract [10], which leads to activation of the NLRP3-inflammasome. In fact, nontypeable *Haemophilus influenzae*-induced inflammation in respiratory cells and tissues results in the upregulation of NOD-like receptor pyrin domain-containing 3 (NLRP3)-inflammasome [11]. This finding suggests a certain role of the activation of NLRP3-inflammasome in the pathogenesis of AECOPD.

Diesel exhaust particles (DEPs) produced by diesel engine vehicles is an important constituent of particulate matter air pollution [12,13]. Because of the large number

of hazardous chemicals present, inhalation of DEPs likely exert harmful effects on the airways of COPD patients. These harmful effects are suggested by the findings of increased secretion of proinflammatory cytokines and reactive oxygen species induced by DEPs [14,15]. DEP exposure leads to elevation of reactive oxygen species, which can activate the NLRP3-inflammasome [16]. Therefore, we speculated that DEP-induced activation of NLRP3-inflammasome may be related to the pathogenesis of AECOPD.

In the present study, we investigated whether the NLRP3-inflammasome is activated in an elastase-induced emphysema model by challenge with DEPs, and whether antioxidants inhibit DEP-induced activation of the NLRP3-inflammasome *in vitro*.

METHODS

Cell culture

RAW 264.7 cells were cultured at 37°C in 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM) medium (Gibco, Langley, OK, USA) supplemented with 10% heat-inactivated fetal calf serum (Gibco), 50 IU/mL penicillin, and 100 mg/mL streptomycin. Cells were subcultured every 3 to 4 days and seeded at 10⁶/mL in six-well plates (Nalge Nunc, Rochester, NY, USA).

Animals

Experiments protocols were performed according to the Guidelines for the Care and Use of Laboratory Animals of the National Health Institute, and were approved by the Animal Ethics Committee of Soon Chun Hyang University Bucheon Hospital (SCHBC-animal-201409). C57BL/6 female mice, at 12 weeks of age, were purchased from Central Lab Animal Inc. (Seoul, Korea) and were used in the present study. The animals were housed in a temperature- and humidity-controlled room with free access to water and standard laboratory food. Mouse pulmonary emphysema was induced by one-time intratracheal administration of 15 μ g of porcine pancreatic elastase (PPE) (E788, Sigma-Aldrich, St. Louis, MO, USA) in 50 μ L of phosphate-buffered saline (PBS) while control mice received 50 μ L of sterile PBS [17]. After 3 weeks of intratracheal instillation, animals were euthanized. Each group consisted of 12 mice, of which six were used

for lung histology and bronchoalveolar lavage (BAL) and the other six for *ex vivo* explants culture. For the explants culture, six pieces of lung tissues (20 to 30 mg/each) were treated similarly as the cell culture.

Preparation of cigarette smoking extract

The cigarette smoking extract (CSE) was produced using a modification of the method of Smelter et al. [18]. Briefly, a 50-mL plastic syringe was attached to the smoking apparatus (Smoking Tester System, Three Shine Inc., Daejeon, Korea) in the atmosphere. Smoke (35 mL) from Korean cigarettes (THIS, KT&G, Seoul, Korea) were slowly drawn into the attached syringe and passed through 30 mL of cell culture medium (DMEM). The CSE solution was then filtered and used within 30 minutes. The concentration of the CSE solution thus produced was considered to be 100%.

In vitro cell culture

After 18 hours of starvation, cells were stimulated with various concentrations of CSE (5% to 30%), DEPs (5% to 30%) (Diesel Particular Matter; Standard Reference Material 1650b; average particle diameter 0.18 μm ; surface area, 108 m^2/g ; National Institute of Standards and Technology, Gaithersburg, MD, USA), or 1 mg/mL lipopolysaccharide (LPS) as the positive control. The concentration and stimulation time of CSE and DEPs were

analyzed by measuring the IL-1 β level in the culture supernatant of RAW 264.7 cells. The IL-1 β concentration was maximal at the 10% dilution after 24 hours of stimulation with CSE and DEP (Fig. 1). RAW cells and elastase-treated lung explants were stimulated with 10% CSE, DEPs, and 1 mg/mL LPS with or without 10 μM N-acetylcysteine (NAC) or 100 μM of the caspase-1 inhibitor, Z-YVAD-FMK (Calbiochem, Darmstadt, Germany).

Bronchoalveolar lavage

BAL was performed as described previously [19]. Briefly, BAL fluid was obtained by instilling a total volume of 1.5-mL saline into the lungs three times through a tracheal tube. The BAL fluid was centrifuged (150 $\times\text{g}$, 10 minutes, 4°C), and the cell pellet was resuspended in 1 mL of PBS. A 50- μL volume of the suspension was mixed with the same volume of 4% trypan blue to determine the cell numbers and viability. To perform differential cell counts in BAL fluid, 5×10^3 cells were mounted on a slide by cytocentrifugation and stained with Diff-Quik (Baxter Healthcare, Miami, FL, USA).

Tissue preparation and fixation

Following BAL, the chest wall was opened. The lungs were inflated with 10% phosphate-buffered formalin at a transpulmonary pressure of 25 cmH_2O for 1 hour. The tissues were embedded in paraffin wax and were cut for

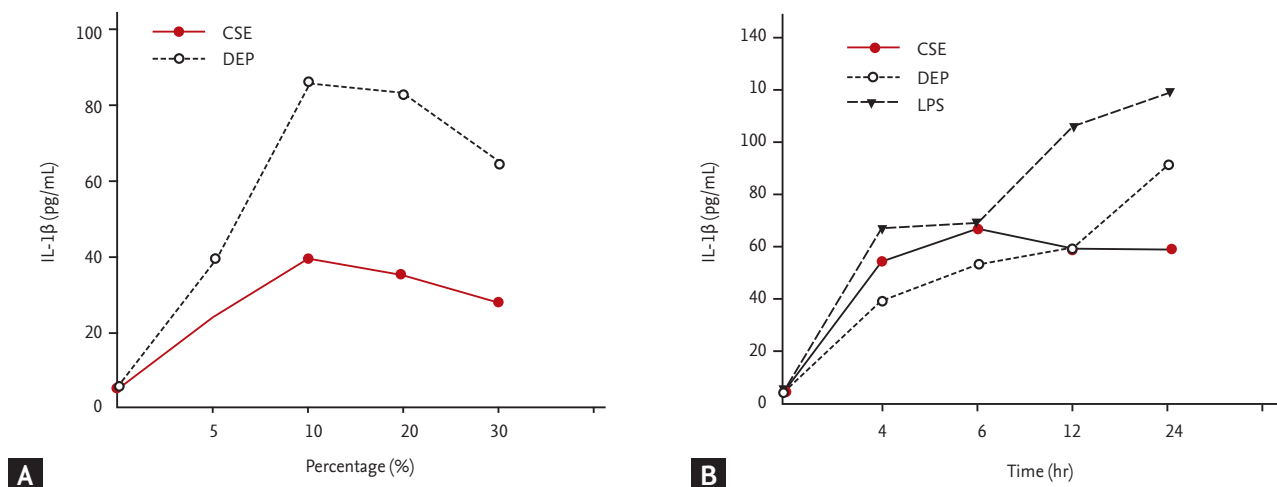


Figure 1. (A) Interleukin-1 β (IL-1 β) secretion by RAW 264.7 cells after 24-hour incubation with various concentrations of cigarette smoking extract (CSE) and diesel exhaust particles (DEPs). IL-1 β secretion peaked at a 1:10 dilution of both CSE and DEPs. (B) IL-1 β secretion by RAW 264.7 cells at 4 to 24 hours in the presence of a 1:10 dilution of CSE, DEPs, or 1 mg/mL lipopolysaccharide (LPS). IL-1 β secretion peaked at 24 hours for LPS and DEPs; however, CSE resulted in a plateau after 6 hours. The data shown are representative of three independent experiments.

H&E staining. Cultured lung tissue explants were embedded in paraffin wax blocks and were cut for immunohistochemical staining for NLRP3 and caspase-1.

IL-1 β assay

Cell and tissue culture supernatants were collected by centrifugation to remove cellular debris. Supernatants were stored as aliquots at -70°C until use. IL-1 β was assayed using a Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA), as described by the manufacturer.

Mean linear intercept

To calculate the change in alveolar destruction, we measured the air space size using the mean linear intercept (MLI) according to a modified method described previously [20]. Briefly, after paraffin embedding, $5\text{-}\mu\text{m}$ sections were cut and stained with H&E. Ten randomly selected fields under the magnification of $100\times$ were assessed from each lung. Fifteen lines were randomly placed on the lung sections, and the number of intercepts crossing the alveolar wall was counted. The MLI was calculated from the length of the lines multiplied by the number of the lines divided by the sum of all counted intercepts.

Immunohistochemistry

A VectaStain rabbit ABC Elite kit (Vector Laboratories, Burlingame, CA, USA) was used for immunostaining. Tissue sections ($5\ \mu\text{m}$) were deparaffinized, endogenous peroxidase was blocked with 1.4% H_2O_2 in methyl alcohol for 30 minutes, and nonspecific binding was blocked with 1.5% normal rabbit serum for 30 minutes. The sections were incubated with rabbit polyclonal anti-mouse NLRP3-inflammasome antibody ($1:500$; Abnova, Taipei, Taiwan) at 4°C for 16 hours. After washing with Tris-buffered saline (TBS), the sections were sequentially incubated with biotinylated rabbit anti-rabbit immunoglobulin G (H+L) ($1:200$; Vector Laboratories) and avidin-biotin peroxidase complex ($1:50$; ABC kit, Vector Laboratories) for 30 minutes. The color reaction was developed with $3,3'$ -diamino-benzidine tetrachloride (Zymed Laboratories, South San Francisco, CA, USA), and Harris hematoxylin was applied as a counter stain.

Western blotting

A $10\ \mu\text{g}$ of protein from lung tissue sample was electro-

phoresed on 15% polyacrylamide gels using a discontinuous system. The proteins were transferred to nitrocellulose membranes at $120\ \text{V}$ for 40 minutes. After blocking in 5% skim milk and 0.1% NP40 in TBS for 2 hours at room temperature, the membranes were incubated with a rabbit polyclonal anti-mouse NLRP3-inflammasome (Abnova) or rabbit monoclonal anti-mouse caspase-1 (Abcam, Cambridge, MA, USA) primary antibody. The membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit immunoglobulin G ($1:2,000$ dilution) for 1 hour at room temperature. The target protein was detected using an enhanced chemiluminescence reagent (Amersham Pharmacia Biotech, Little Chalfont, UK).

RESULTS

IL-1 β secretion by RAW 264.7 cells after stimulation with CSE and DEPs

IL-1 β concentrations in the supernatants of RAW 264.7 cells were measured after stimulation with 10% CSE and DEPs (Fig. 1). CSE and DEP exposure led to marked IL-1 β secretion. To evaluate the effects of oxidants and caspase-1 on IL-1 β secretion, cells were incubated

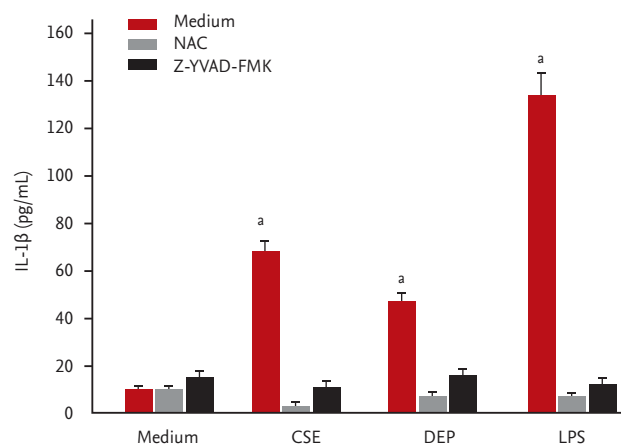


Figure 2. Interleukin-1 β (IL-1 β) secretion by RAW 264.7 cells after 24-hour stimulation with cigarette smoking extract (CSE) and diesel exhaust particles (DEPs) in the presence or absence of N-acetylcysteine (NAC) or a caspase-1 inhibitor. CSE-, DEP-, and lipopolysaccharide (LPS)-induced increases in IL-1 β levels in the supernatants of RAW 264.7 cells were significantly decreased by NAC and Z-YVAD-FMK. ^a $p < 0.01$ vs. NAC and Z-YVAD-FMK.

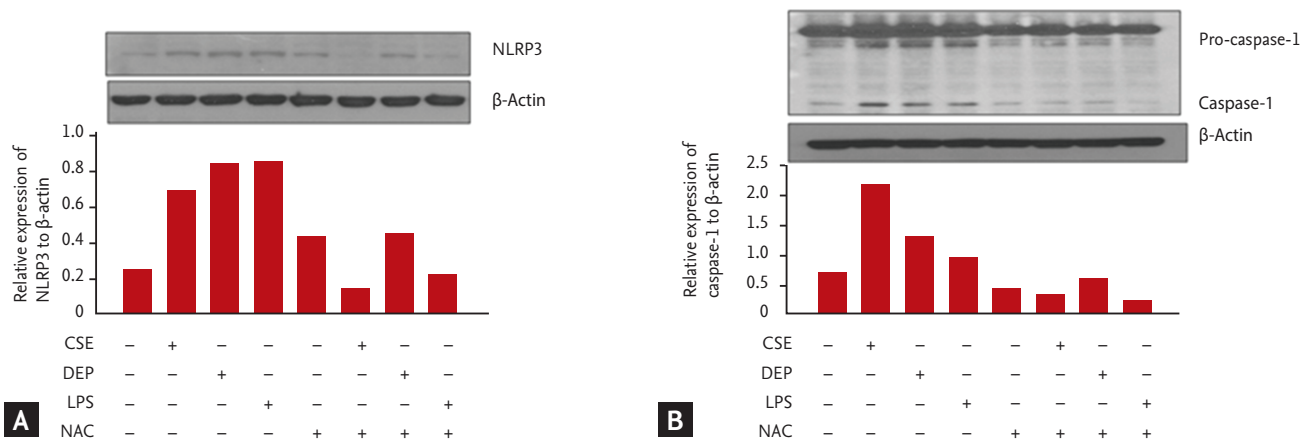


Figure 3. Western blot analysis of (A) nucleotide-binding oligomerization domain-like receptor (NLR) family members containing the pyrin domain (NLRP3)-inflammasome and (B) caspase-1 levels after stimulation with cigarette smoking extract (CSE) and diesel exhaust particles (DEPs). (A) NLRP3-inflammasome levels in RAW 264.7 cells were increased following stimulation with CSE and DEP for 24 hours, but were decreased by N-acetylcysteine (NAC) application. (B) Caspase-1 levels in RAW 264.7 cells were increased following stimulation with CSE and DEPs for 24 hours. This expression was decreased by NAC. Lipopolysaccharide (LPS) was used as positive control.

with the antioxidant NAC and the caspase-1 inhibitor Z-YVAD-FMK for 24 hours. The IL-1 β concentrations were decreased significantly in the supernatants of RAW 264.7 cells by the antioxidant and the caspase-1 inhibitor ($p < 0.05$) (Fig. 2).

NLRP3-inflammasome and caspase-1 protein levels in RAW 264.7 cells after stimulation with CSE and DEPs

We evaluated whether NLRP3-inflammasome and caspase-1 levels in RAW 264.7 cells were upregulated by stimulation with CSE and DEPs by Western blotting. NLRP3-inflammasome expression was increased by stimulation with DEPs and CSE and inhibited by the antioxidant NAC (Fig. 3A). Caspase-1 expression was up-regulated by CSE and DEPs and decreased in the presence NAC (Fig. 3B).

Cellular profiles, histology, and morphometric analysis of an elastase-induced emphysema model

The total cell count in BAL fluid was not different between the control group and elastase-induced emphysema group. The lymphocyte numbers significantly increased in the emphysema group compared with the control group, but the numbers of other cell types were not different between the two groups (Fig. 4). The architecture of

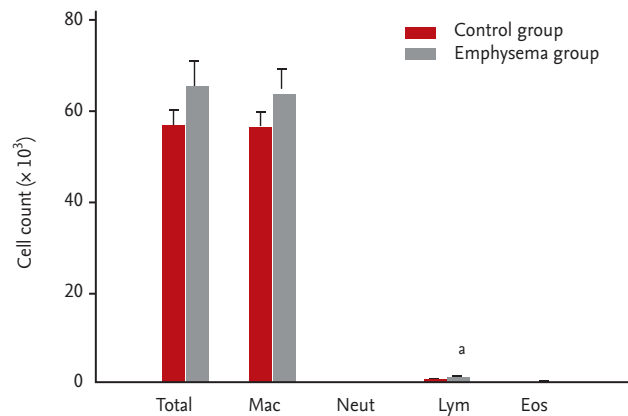


Figure 4. Total cell and inflammatory cell counts in bronchoalveolar lavage fluid from the control and emphysema groups. Lymphocyte numbers were significantly increased in the emphysema group compared with the control group. The numbers of other cell types were not different between the two groups. Total, total cell count; Mac, macrophages; Neut, neutrophils; Lym, lymphocytes; Eos, eosinophils. ^a $p < 0.05$ vs. the control group.

alveolar septa and the airspace was well preserved in the control group (Fig. 5A). However, in the elastin-induced emphysema group, alveoli were enlarged (Fig. 5B). The MLI was significantly higher in the elastin-induced emphysema group than in the control group ($90.4 \pm 6.7 \mu\text{m}$ vs. $23.6 \pm 2.2 \mu\text{m}$, $p < 0.01$) (Fig. 5C).

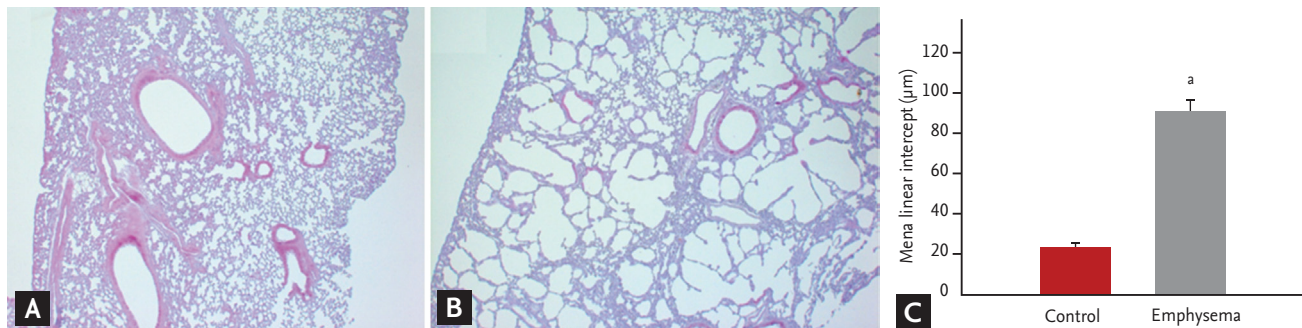


Figure 5. (A, B) Hematoxylin-eosin staining of the lung ($\times 40$) and (C) the mean linear intercepts of both groups. (A) The architecture of alveolar septa and the airspace was well preserved in the control group, and (B) the alveoli were enlarged in the elastin-induced emphysema group. (C) The mean linear intercept was significantly higher in the elastin-induced emphysema group than that in the control group. ^a $p < 0.01$ vs. the control group.

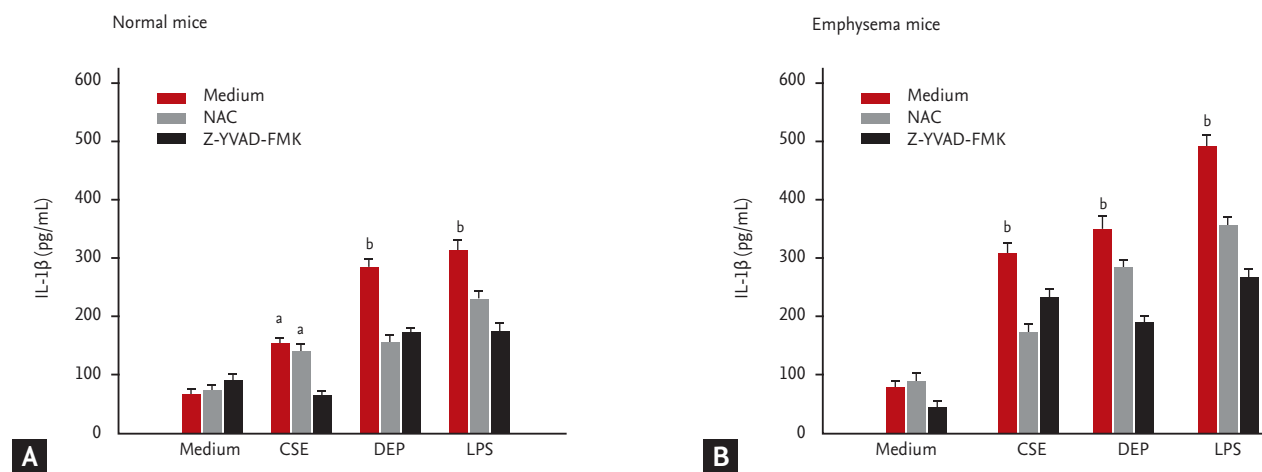


Figure 6. Interleukin-1 β (IL-1 β) secretion from lung tissues after 24 hours stimulation with cigarette smoking extract (CSE) and diesel exhaust particles (DEPs). (A) CSE and DEP exposure increased IL-1 β secretion in both the normal and (B) elastin-induced emphysema groups. N-acetylcysteine (NAC) and Z-YVAD-FMK inhibited the NAC- and DEP-induced IL-1 β secretion in both the normal and elastin-induced emphysema groups. LPS, lipopolysaccharide. ^a $p < 0.05$ vs. Z-YVAD-FMK, ^b $p < 0.05$ vs. NAC and Z-YVAD-FMK.

IL-1 β secretion from lung tissue explants after stimulation with CSE and DEPs

Murine lung tissue explants from the normal and elastase-induced emphysema groups were incubated with CSE and DEPs for 24 hours; the IL-1 β levels in supernatants were then measured. Similar to RAW 264.7 cells, CSE and DEPs increased the secretion of IL-1 β by the cultured lung tissue explants obtained from the normal (Fig. 6A) and elastase-induced emphysema groups (Fig. 6B). IL-1 β secretion by CSE and DEPs increased more in the elastase-induced emphysema group than in the normal group (CSE: 309 ± 19 pg/mL vs. 151 ± 13 pg/mL, respectively, $p < 0.05$; DEP: 350 ± 24 pg/mL vs. 281 ± 15 pg/mL, respectively, $p < 0.05$). NAC and Z-YVAD-FMK

significantly inhibited the NAC- and DEP-induced IL-1 β secretion in both the normal and elastase-induced emphysema groups (Fig. 6).

Immunohistochemical staining of the NLRP3-inflammasome in lung tissues after stimulation with CSE and DEPs

NLRP3-inflammasome expression was detected in lung tissue explants after 24 hours of stimulation with CSE and DEP. The NLRP3 expression was increased by CSE and DEP in both the normal (Fig. 7A) and elastase-induced emphysema group (Fig. 7B). This expression was suppressed by NAC in both the normal and emphysema groups.

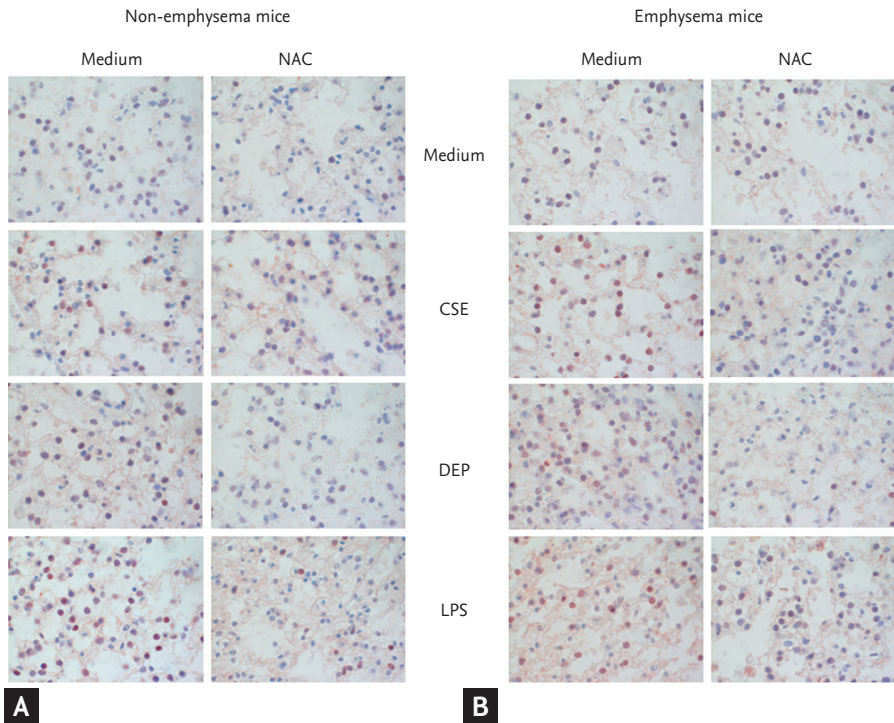


Figure 7. Immunohistochemical staining of the nucleotide-binding oligomerization domain-like receptor (NLR) family members containing the pyrin domain 3 (NLRP3)-inflammasome in lung tissues after 24-hour stimulation with cigarette smoking extract (CSE) and diesel exhaust particles (DEPs) ($\times 100$). (A) NLRP3-inflammasome expression was increased by CSE and DEPs in both the normal and (B) elastin-induced emphysema groups. NLRP3-inflammasome expression was suppressed by N-acetylcysteine (NAC) in both the normal and emphysema groups. LPS, lipopolysaccharide.

DISCUSSION

The present study showed that the NLRP3-inflammasome was upregulated by DEPs and/or CSE, and this upregulation was inhibited by the antioxidant NAC. The exact role of NLRP3-inflammasome is unclear in the pathogenesis of AECOPD, but oxidative stress may be involved because the antioxidant NAC inhibited the DEP-induced increase in IL-1 β levels in a macrophage cell line, and because the NLRP3-inflammasome overexpressed in the elastase-induced emphysema model. Other animal studies have suggested a relationship between DEPs and oxidative stress. Intratracheal instillation of DEPs increased the levels of a superoxide producer (nicotinamide adenine dinucleotide phosphate cytochrome P-450 reductase) and decreased those of a superoxide scavenger (superoxide dismutase) in mice [21]. Although few reports have been published regarding the direct demonstration of inflammasome activation in the lung tissues from patients with COPD at acute exacerbation, activation of NLRP3-inflammasome is likely a key player in the pathogenesis of acute exacerbations. The NLRP3-inflammasome was upregulated during nontypeable H. influenzae-induced inflammation in respiratory cells and human lung tissues [11]. In the present study, we found that the NL-

RP3-inflammasome was upregulated by treatment with DEPs in the lung tissue explants of murine elastase-induced emphysema, which mimics human emphysema. These findings suggest the NLRP3-inflammasome play an important role in DEP-induced inflammation in elastase-induced emphysema animal model.

It is suggested that a two-step reaction is required for the full activation of NLRP3-inflammasome [22,23]. First step is 'priming' which upregulate pro-IL-1 β , pro-IL-18, and the components of inflammasome. Second step is 'activation' which is the assembly of the components into inflammasome structure and production of proinflammatory interleukins. The exact mechanism of activation of inflammasome is unknown, but, a lot of stimuli like microbes and microbial substances, LPS, are found to activate NLRP3 in the presence of adenosine triphosphate [24]. By these reasons, we used LPS as positive control for activation of NLRP3 in the present study.

Air pollution is a known precipitating factor of AECOPD [25,26]. Epidemiologic studies have supported the role of air pollutants, such as nitrogen dioxide (NO $_2$), sulfur dioxide (SO $_2$), particulate matter smaller than 10 μ g (PM $_{10}$), and black smoke particulate matter, in the increase in respiratory symptoms and mortality rate in COPD patients [27]. Exposure of healthy persons

to diesel exhaust results in an increased number of neutrophils in sputum [28], and an *in vitro* study showed an increase in the levels of IL-1 and neutrophil-related cytokines, such as IL-8 and granulocyte-macrophage colony-stimulating factor, in human airway epithelial cells [29,30]. Urban particulate matter including dust and emission particles has been shown to activate the NLRP3-inflammasome in airway epithelium [31]. In the present study, the IL-1 β level was increased by treatment with DEPs in both cell lines and an elastase-induced emphysema model. These findings are compatible with alterations in IL-1 β levels in H1N1 virus-associated AECOPD in animal models [32] and in a cohort of patients with COPD [33].

Intratracheal administration of PPE can induce acute lung inflammation due to increased secretion of inflammatory cytokines, such as IL-1 β and IL-6, during the acute stage [34]. However, in the present study, we euthanized the mice at 3 weeks after intratracheal instillation of elastase, and the neutrophil count in BAL was similar in the emphysema and saline-treated groups. Therefore, elastase had no marked effect on the expression of the NLRP3-inflammasome and lung inflammation.

Our data demonstrated that CSE increased IL-1 β secretion in the emphysema model; however, in the control group, IL-1 β secretion did not attain the level reached in the emphysema mode (Fig. 6). This finding may be due to secretion of various cytokines by activated alveolar macrophages following cigarette smoke exposure [35]. We expected DEPs to show a pattern of IL-1 β secretion similar to CSE; however, DEPs increased IL-1 β levels to a degree similar to that in both the emphysema and non-emphysema models (Fig. 6). A study involving intratracheal instillation of DEPs in an elastase-induced emphysema model showed no difference in the IL-1 β level between the emphysema and control groups [34], which is in agreement with our findings.

Caspase-1 levels are increased in the lung tissues of patients with COPD compared with those from nonsmokers [36], serving as indirect evidence of the role of the inflammasome in the pathogenesis of COPD. However, no reports involving direct measurement of inflammasome expression in COPD lung tissues are available. Thus, in the present study, the elevated inflammasome levels induced by DEPs in both epithelial cell lines and an elastase-induced emphysema model may be related

more closely to AECOPD than its pathogenesis.

The limitations of this study were as follows. First, experiments using NAC should be interpreted with caution. NAC may detoxify other reactive DEP constituents that could trigger inflammatory reactions and suppress reactive oxygen species, which are important mediators in most signal transduction pathways [37,38]. Thus DEP-induced overexpression of the NLRP3-inflammasome may be related to factors other than oxidative stress. Second, the normal ambient DEP concentrations are typically lower than those used in this study. We attempted to generate model of DEP-induced AECOPD, but inducing lung inflammation using a low concentration of DEPs is problematic. Other studies with human volunteers have also used a DEP concentration higher than the ambient [39,40]. Third, we did not measure lung mechanical parameters such as enhanced pause (Penh) and total respiratory systemic resistance. Although we did not measure lung mechanics, the increased MLI could be a good indicator of emphysema. Fourth, the concentration of DEP used in the present study is higher than those in ambient air. We tried to reduce the dose of DEP in the present study, but we should choose higher concentration of DEP by the finding of the dose-response curve shown in Fig. 1.

In conclusion, these data suggest that the NLRP3-inflammasome was activated by challenge with DEPs in an elastase-induced emphysema model. Furthermore, an antioxidant inhibited the DEP-induced activation of the NLRP3-inflammasome.

KEY MESSAGE

1. The nucleotide-binding oligomerization domain-like receptor (NLR) family members containing the pyrin domain 3 (NLRP3)-inflammasome is activated by diesel exhaust particles in *ex vivo* tissue explants from elastase-induced emphysema animal model, and this activation is inhibited by N-acetylcysteine.
2. Diesel exhaust particles could be an important cause of acute exacerbation of chronic obstructive pulmonary disease through activation of NLRP3-inflammasome.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

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