ALTERED PROINFLAMMATORY CYTOKINES AND M1 POLARIZATION INDUCED BY PM2.5 IN ALVEOLAR MACROPHAGES

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Abstract. Exposure to atmospheric particulate matter with aerodynamic diameter less than 2.5 μm (PM2.5) is epidemiologically implicated in pulmonary mortality and diseases. While it is known that PM2.5-exposed macrophages produce and secrete inflammatory cytokines, it is not clear how PM2.5 contributes to the upregulation of proinflammatory cytokines in alveolar macrophages. Therefore, the present study aims to investigate the molecular mechanism of macrophage inflammatory responses to PM2.5. In a MH-S mouse alveolar macrophage cell line, exposure to PM2.5 significantly increased the intracellular levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) in dose- and time-dependent manners, while it did not alter those of transforming growth factor-β1 (TGF-β1) and interleukin-10 (IL-10). PM2.5 also increased the expressional level of CD80 mRNA, but did not alter that of CD163 mRNA in alveolar macrophages, suggesting PM2.5-induced M1 phenotypic polarization. Treatment with nuclear factor-κB (NF-κB) inhibitors immediately after PM2.5 administration reduced the production of TNF-α, IL-1β, and IL-6 upregulated by PM2.5, and attenuated the CD80 mRNA expression induced by the PM2.5 exposure. In conclusion, these data suggest that PM2.5 exposure in alveolar macrophages induces the upregulation of proinflammatory cytokines as well as polarization to the M1 phenotype through the NF-κB activation.
Keywords: *air pollution, Jeju Island, lung, CD80, NF-κB*

Introduction

Global air pollution has a major impact on the health of humans and organisms' lives as well as the natural environment (Kampa and Castanas, 2008). Among the primary air pollutants including particulate matter (PM), carbon monoxide, lead, nitrogen dioxide, ozone, and sulfur dioxide, PM is a complex mixture of airborne solid particles and liquid droplets, consisting of acids, organic chemicals, metals, and dust particles

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(Anderson et al., 2012). In several epidemiological studies, the elevated level of PM is estimated to be associated with increased mortality and hospitalization (Kim et al., 2015b). Especially, because PM2.5 with an aerodynamic diameter of less than 2.5 μm contains various toxic substances and penetrates deeply into the lungs, considerable studies have shown that PM2.5 triggers severe respiratory diseases including bronchitis, asthma, chronic obstructive pulmonary disease, and lung cancer as well as mild problems including chest pain, coughing, and wheezing (Abbey et al., 1995; Ko et al., 2007; Tecer et al., 2008; Vinikoor-Imler et al., 2011). Experimental laboratory evidence suggests that exposure to PM2.5 induces the production of proinflammatory cytokines, the generation of reactive oxygen species, and alteration in macrophage polarization during lung injury (Deng et al., 2013; Riva et al., 2011; Zhao et al., 2016). Nevertheless, studies on the relationship between PM2.5 and the inflammatory response in alveolar macrophages are still limited.

The alveolar macrophage is a tissue-resident phagocyte just as Kupffer cells in the liver, Langerhans cells in the skin, osteoclasts in bone, and microglial cells in the central nervous system. The alveolar macrophage is the only macrophage living in aerobic conditions; and constitutes the first line of phagocytic defense against inhaled particles and microbial agents in the alveolar space (Hocking and Golde, 1979). In particular, these cells play a critical role in lung defense systems through their ability to scavenge the inhaled particles and microbial agents that infiltrate the gas-exchanging airway, recruit polymorphonuclear leukocytes from the pulmonary vasculature into the alveolar spaces, and regulate the immune response. Although the crucial proinflammatory mediators produced by alveolar macrophages, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), are beneficial to lung defense in vivo, the excessive production of these cytokines is involved in the pathogenesis of inflammatory and allergic lung diseases, which can result in lung dysfuction. Previous studies have often shown the induction of proinflammatory cytokines by exposure to PM2.5 in RAW264.7 macrophages derived from mouse peripheral blood (Jalava et al., 2007) and THP-1 monocytes derived from human peripheral blood (Corsini et al., 2013; Zhang et al., 2018), but have not shown that in alveolar macrophages. Some investigators have used primary cultures of alveolar macrophages derived from lungs of humans and rats for studies on the proinflammatory activity of PM2.5 (Hetland et al., 2005; Soukup and Becker, 2001b); however, the application of primary alveolar macrophage cultures in experimental studies is difficult due to the instability of their phenotype and function ex vivo (Wang et al., 2013). Furthermore, polarized macrophages including alveolar macrophage can be commonly classified into two main phenotypes (Martinez et al., 2008). Between both types, M1 macrophages polarized by Th1-type cytokines and pathogens promote inflammation through upregulating proinflammatory cytokines, and increase the production of reactive oxygen species and nitrogen intermediates. In contrast, M2 macrophages are closely related to the Th2 type and immunomodulatory cytokines, which primarily suppress inflammation (Gordon, 2007). However, the precise mechanisms of the proinflammatory cytokine induction and the polarization in alveolar macrophages by PM2.5 are still unclear.

Nuclear factor-κB (NF-κB) transcription factors are known to play an important role in immune and inflammatory responses (Lenardo and Baltimore, 1989). The activation of the NF-κB signaling pathway requires the signal transduction of external stimuli including bacterial lipopolysaccharide, oxidants, viruses, and cytokines. Especially, the level of proinflammatory cytokines including TNF-α, IL-1β, and IL-6 is regulated by

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NF-κB, while these cytokines stimulate the activation of NF-κB and induce a cascade of proinflammation. Although PM2.5 has been shown to activate NF-κB in alveolar epithelial cells and peripheral macrophages (Dagher et al., 2007; He et al., 2017), the involvement in NF-κB signaling induction by PM2.5 in alveolar macrophages remains unknown. Therefore, we attempted to determine whether the PM2.5-induced upregulation of cytokines is dependent on the NF-κB signaling pathway in the HM-S alveolar macrophages derived from mouse lungs. In addition, to clarify the mechanism of PM2.5-induced lung inflammation, we evaluated whether PM2.5 acts directly on alveolar macrophage polarizations under the cell culture condition without any inducing systems for macrophage polarization.

Materials and methods

PM2.5 preparation and chemical analysis

PM2.5 was collected by the Jeju Special Self-Governing Province Research Institute of Health & Environment during September 2017 from a measuring station at latitude 33°29'19.2" north and longitude 126°30'00.5" east in Yeondong, which is located in the northern area of Jeju Island, Republic of Korea. The samples were performed on quartz filters (Millipore, Bedford, MA, USA) for biological analysis and polytetrafluoroethylene (PTFE) fiber membrane filters (Millipore) for chemical analysis using a PM2.5 sequential sampler (PMS-103, APM, Bucheon, Korea). To carry out biological studies using the PM2.5, the quartz filters were detached from plastic containers with care to avoid material loss or contamination, weighted using a microbalance (XP6, Mettler Toledo, Columbus, OH, USA) to calculate the weight of PM2.5, immersed in Roswell Park Memorial Institute (RPMI) 1640 culture medium (Welgene, Daegu, Korea) at a final concentration of 100 μg/ml, and sonicated for 60 min at room temperature in a sonicating water bath (Branson Ultrasonics, Danbury, CT, USA). The PM2.5 extract in RPMI 1640 was stored at -80°C and resuspended just prior to use. To analyze the water soluble components in the PM2.5, the halves of PTFE filters were exposed to constant temperature (20°C) and humidity (45%) for 24 h, weighted using a microbalance (XP6, Mettler Toledo) to calculate the weight of PM2.5, soaked in 100 μl ethanol, immersed in 10 ml distilled water, sonicated for 60 min, and filtrated using a 0.45 μm polyvinylidene fluoride (PVDF) syringe filter. The PM2.5 extract in 1% ethanol was used to measure the water soluble components including Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, NH^{4+}, Cl^{-}, NO_3^{-}, and SO_4^{2-} using an ion chromatography (Dionex ICS-2000, Dionex, Sunyvale, CA, USA). To analyze the elements in the PM2.5, the other halves of PTFE filters were exposed to constant temperature (20°C) and humidity (45%) for 24 h, weighted using a microbalance (XP6, Mettler Toledo) to calculate the weight of PM2.5, digested with 10 ml solvent (5.55% HNO_3 + 16.75% HCl) in a microwave digestion system (ETHOS One, Milestone, Sorisole, Italy) at 200°C for 30 min, supplemented with 10 ml distilled water and filtrated using a 0.45 μm PVDF syringe filter. The PM2.5 extract in the solvent was used to measure the elements including Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, Ti, U, V, and Zn using an inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7800, Agilent Technologies, Tokyo, Japan). *Table 1* shows the results of chemical analysis.

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Table 1. *Concentrations (μg/mg PM2.5/day) of elements and water soluble components in PM2.5*

Element (mean ± s.d.)		Water soluble component (mean ± s.d.)	
Al	4.58 ± 1.14	Ca^{2+}	2.86 ± 0.92
As	0.14 ± 0.07	K^{+}	7.47 ± 3.72
Ba	0.29 ± 0.04	Mg^{2+}	1.66 ± 0.60
Ca	3.77 ± 0.34	Na^{+}	7.09 ± 3.56
Cd	0.03 ± 0.02	NH_4^{+}	139.25 ± 75.61
Co	0.01 ± 0.01	Cl^{-}	1.04 ± 0.69
Cr	0.31 ± 0.27	NO_3^{-}	2.94 ± 1.97
Cu	0.20 ± 0.07	SO_4^{2-}	430.59 ± 257.63
Fe	7.53 ± 3.30		
K	7.54 ± 3.80		
Mg	5.07 ± 0.89		
Mn	0.38 ± 0.16		
Mo	0.04 ± 0.03		
Na	27.16 ± 2.76		
Ni	0.69 ± 0.97		
Pb	0.66 ± 0.34		
Se	0.11 ± 0.08		
Sr	n.d.		
Ti	0.41 ± 0.10		
U	n.d.		
V	0.38 ± 0.18		
Zn	2.74 ± 0.75		

Ca^{2+}, calcium cation; K^{+}, potassium cation; Mg^{2+}, magnesium cation; Na^{+}, sodium cation; NH^{4+}, ammonium cation; Cl^{-}, chloride ion; NO_3^{-}, nitrate ion; SO_4^{2-}, sulfate ion; Al, aluminum; As, arsenic; Ba, barium; Ca, calcium; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; Mo,molybdenium; Na, sodium; Ni, nickel; Pb, lead; Se, selenium; Sr,strontium; Ti, titanium; U, uranium; V, vanadium; Zn, zinc; n.d., not detectable

Cell culture and treatment

The HM-S mouse alveolar macrophage cell line (American Type Culture Collection, Rockville, MD) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Welgene) at 37°C with 5% CO_2 as previously described (Kim, 2016; Park et al., 2015; Song et al., 2016; Yoon and Kim, 2016). The cells were grown until 70% confluence on 60-mm cell culture dishes. After that, the culture was treated with PM2.5 at a final concentration of 0, 1, 3, 10, or 30 mg/ml for 0, 1, 3, 6, or 24 h. Some cells were treated with either Ro 106-9920 (1 to 10 μM), pyrrolidinedithiocarbamate ammonium (PDTC, 10 to 100 μM), or vehicle (0.1% dimethyl sulfoxide, DMSO) immediately after PM2.5 administration. The selective nuclear factor-κB (NF-κB) inhibitor Ro 106-9920 and PDTC were purchased from R&D Systems (Minneapolis, MN, USA).

Enzyme-linked immunosorbent assay (ELISA)

The levels of TNF-α, IL-1β, IL-6, transforming growth factor-β1 (TGF-β1) and interleukin-10 (IL-10) production were measured in 10 μg protein in 10 μl cell lysates using multiplex immunoassay (Millipore) as previously described (Kim, 2017a; Kim et

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al., 2014; Kim et al., 2015a; Yoon and Kim, 2015). The DNA-binding activity of NF-κB p65 was measured in the nuclear extracts of cells using the TransAM NF-κB p65 transcription factor assay kit (Active Motif, Carlsbad, CA, USA). All ELISA experiments were rigorously performed according to respective manufacturer's protocols.

Real-time quantitative RT-PCR

Total RNA from cells was extracted using TRIzol reagent (ThermoFisher Scientific, Pittsburgh, PA, USA) and cleaned with RNeasy mini kit (Qiagen, Hilden, Germany). After cDNA synthesis using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA), mRNA levels were evaluated using a SYBR Green master mix on an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA) as previously described (Kim et al., 2012). The following primers were used: mouse cluster of differentiation 80 (CD80; forward: GGCAAGGCAGCAATACCTTA; reverse: CTCTTTGTGCTGCTGATTCG) and mouse cluster of differentiation 163 (CD163; forward: TCCACACGTCCAGAACAGTC; reverse: CCTTGGAAACAGAGACAGGC). Cycle conditions were as follows: after an initial hold of 2 min at 50°C and 10 min at 95°C, the samples were cycled 40 times at 95°C for 15 sec and 60°C for 60 sec. Their levels were normalized to the mouse peptidylprolyl isomerase A signal amplified in a separate reaction (forward: TATCTGCACTGCCAAGACTGAATG; reverse: CTTCTTGCTGGTCTTGCCATTCC).

Western blot

Electrophoresis of 50 μg protein in cell lysates on Any kD Mini-PROTEIN TGX gels (Bio-Rad, Hercules, CA, USA) using tris-glycine buffer systems and subsequent blotting onto PVDF membranes were performed as previously described (Kim, 2017b; Lee et al., 2015; Yoon and Kim, 2018a; Yoon and Kim, 2018b). Membranes were incubated with antibodies against phospho-NF-κB p65 (p-NF-κB p65; 1:2,500 dilution; catalog no. 3033, Cell Signaling, Beverly, MA, USA), NF-κB p65 (1:2,500 dilution; catalog no. 8242, Cell Signaling), and β-actin (1:5,000 dilution; catalog no. A2228; Sigma, St. Louis, MO) overnight at 4°C, respectively. After washing, peroxidase anti-rabbit IgG antibodies (1:5,000 dilution; catalog no. WB-1000; Vector Laboratories, Burlingame, CA, USA) against p-NF-κB p65 and NF-κB p65 antibodies, and peroxidase anti-mouse IgG antibodies (1:5,000 dilution; catalog no. WB-2000; Vector Laboratories) against β-actin antibody were applied for 1 h at room temperature. After that, Western Lighting chemiluminescence reagent (NEL101; PerkinElmer, Boston, MA, USA) was used to detect proteins. The anti-β-actin antibody was used for loading control on stripped membranes. The bands were quantified using AzureSpot analysis software (Azure Biosystems, Dublin, CA, USA).

Statistical analysis

Analysis of variance was used to compare data among groups using Systat SigmaPlot (Systat Software Inc., San Jose, CA, USA). Differences between two groups were assessed by two-tailed unpaired Student's t-tests. P values <0.05 were considered statistically significant.

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Results

PM2.5 upregulates proinflammatory cytokines, but does not alter anti-inflammatory cytokines in alveolar macrophages

To determine whether proinflammatory cytokines are upregulated after exposure to PM2.5 in alveolar macrophages, we measured the protein production of TNF-α, IL-1β, and IL-6 cytokines at 24 h after PM2.5 administration in MH-S cells. PM2.5 significantly increased the levels of these cytokines in a dose-dependent manner (*Fig. 1, a to c*). Especially, the TNF-α and IL-6 productions were more susceptible to a low concentration of PM2.5 (3 μg/ml) than IL-1β production (*Fig. 1, a to c*). Next, we tested whether PM2.5-treated alveolar macrophages produce anti-inflammatory cytokines including TGF-β1 and IL-10. The intracellular levels of those proteins were not significantly altered at 24 h after PM2.5 administration (*Fig. 1, d and e*).

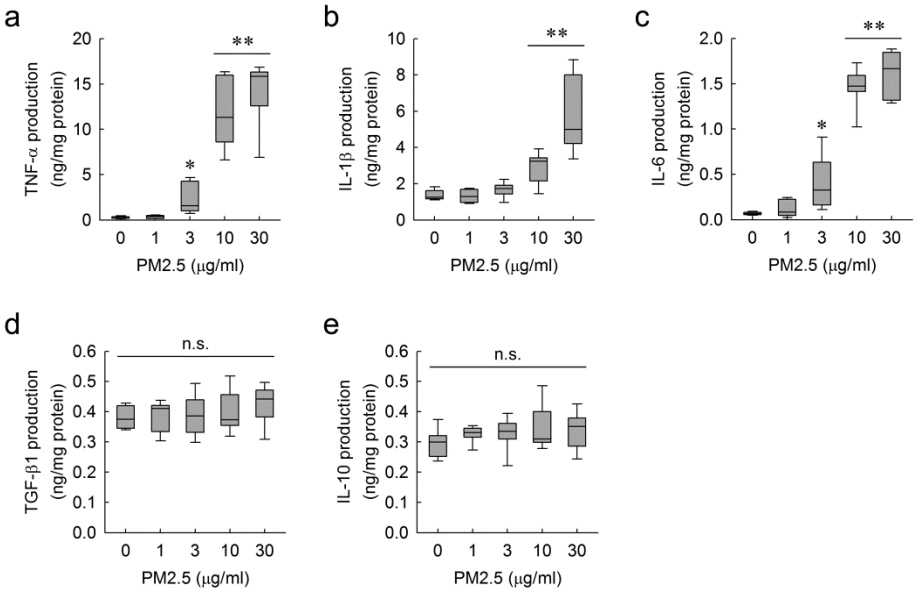

Figure 1. *PM2.5 dose-dependently upregulates proinflammationy cytokines in alveolar macrophages. MH-S cells were treated with 0, 1, 3, 10, or 30 μg/ml of PM2.5 for 24 h (n=9 cultures in each group). Intracellular levels of cytokine TNF-α, IL-1β, IL-6, TGF-β1 and IL-10 were measured using the multiplex immunoassay. In the box plots, whiskers represent the minimum and maximum; boxes represent the interquartile range between the first and third quartiles; and midlines represent the median. *P<0.01 and **P<0.001 versus 0 μg/ml; n.s., not significant*

In addition, to assess the proinflammatory and anti-inflammatory cytokine productions during early exposure to PM2.5 in alveolar macrophages, the protein levels of TNF-α, IL-1β, IL-6, TGF-β1, and IL-10 were measured at 1, 3, and 6 h after PM2.5 administration in MH-S cells. The levels of proinflammatory cytokines including TNF-α, IL-1β, and IL-6 were markedly enhanced during PM2.5 exposure in a time-dependent manner (*Fig. 2, a to c*), but the levels of anti-inflammatory cytokines including TGF-β1 and IL-10 were not significantly altered (*Fig. 2, d and e*). Taken together, these data suggest that PM2.5 specifically induces the upregulation of proinflammatory cytokines in alveolar macrophages.

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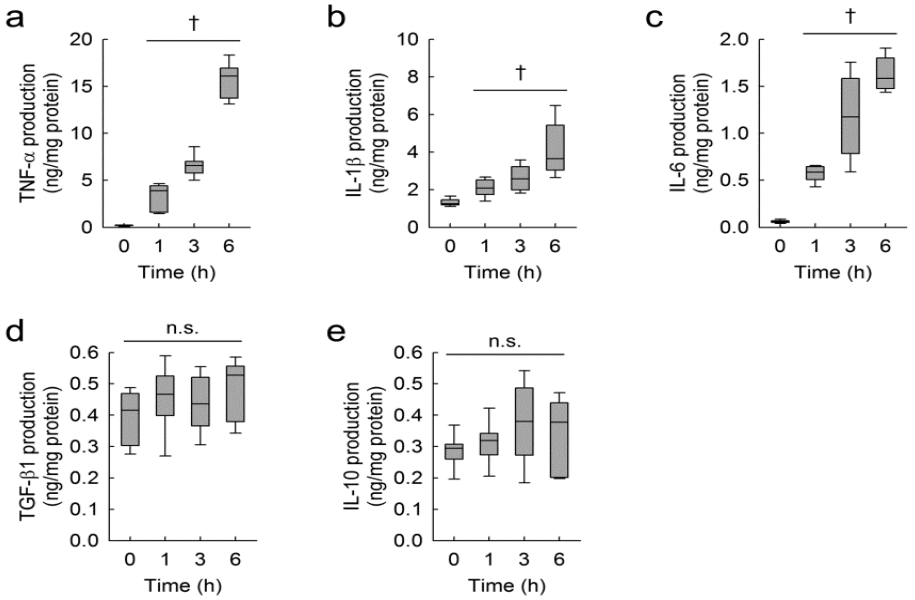

Figure 2. *PM2.5 time-dependently upregulates proinflammationy cytokines in alveolar macrophages. MH-S cells were treated with 30 μg/ml of PM2.5 for 0, 1, 3, or 6 h (n=9 cultures in each group). Intracellular levels of cytokine TNF-α, IL-1β, IL-6, TGF-β1 and IL-10 were measured using the multiplex immunoassay. In the box plots, whiskers represent the minimum and maximum; boxes represent the interquartile range between the first and third quartiles; and midlines represent the median. †P<0.001 versus 0 h; n.s., not significant*

PM2.5 stimulates M1 phenotypic polarization in alveolar macrophages

Generally, macrophages are polarized into two distinct phenotypes: classically activated macrophages (M1) and alternatively activated macrophages (M2) (Sica and Mantovani, 2012). M1 macrophages upregulate proinflammatory cytokines including TNF-α, IL-1β, and IL-6, while M2 macrophages upregulate anti-inflammatory cytokines including TGF-β1 and IL-10 (Arango Duque and Descoteaux, 2014). The results shown in *Fig. 1 and 2* indicate that PM2.5 promotes alveolar macrophage polarization toward a M1-like proinflammatory phenotype. To investigate the phenotypic character of alveolar macrophages during PM2.5 exposure, the mRNA expression of the M1/M2 marker genes was evaluated at 24 h after PM2.5 administration in the MH-S cells. PM2.5 significantly increased the mRNA level of M1 marker CD80 in a dose-dependent manner, while it did not alter the mRNA level of M2 marker CD163 (*Fig. 3, a and b*). Within 24 h after PM2.5 administration, the intracellular levels of CD80 mRNA expression were also increased, while the level of CD163 mRNA expression was not altered (*Fig. 3, c and d*). Therefore, these data suggest that PM2.5 directly stimulates polarization toward a M1 phenotype in alveolar macrophages.

NF-κB inhibition attenuates PM2.5-induced proinflammatory cytokine upregulation in alveolar macrophages

The NF-κB transcriptional factor plays an essential role in the transduction signals involved in the expression of a number of inflammatory genes (Lenardo and Baltimore, 1989) and is activated by PM2.5 in alveolar epithelial cells and peripheral macrophages

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(Dagher et al., 2007; He et al., 2017). As shown in *Fig. 4*, the p65 protein as a major subunit of NF-κB was also dose-dependently activated by exposure to PM2.5 in alveolar macrophages, as measured by the p65 phosphorylation and DNA-binding activity in the MH-S cells at 6 h after PM2.5 administration.

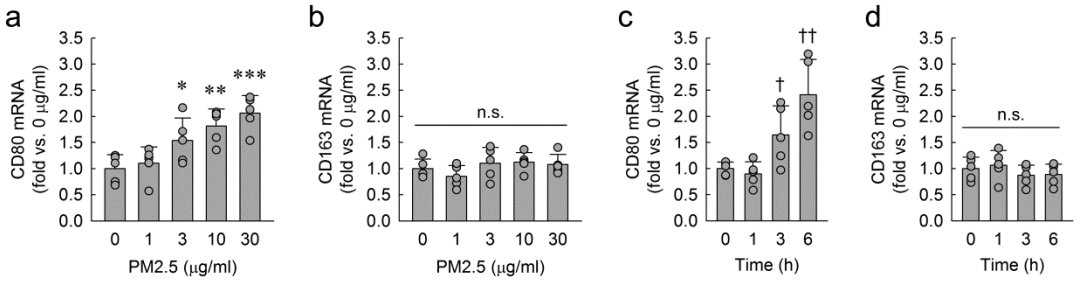

Figure 3. *PM2.5 increases CD80 mRNA expression, but not CD163 mRNA in alveolar macrophages. (a and b) MH-S cells were treated with 0, 1, 3, 10, or 30 μg/ml of PM2.5 for 24 h (n=5 cultures in each group). (c and d) MH-S cells were treated with 30 μg/ml of PM2.5 for 0, 1, 3, or 6 h (n=5 cultures in each group). Intracellular levels of CD80 and CD163 mRNA were measured using real-time quantitative RT-PCR. *P<0.05, **P<0.01, and **P<0.001 versus 0 μg/ml. Data are shown as mean + s.d. †P<0.05 and ††P<0.01 versus 0 h; n.s., not significant*

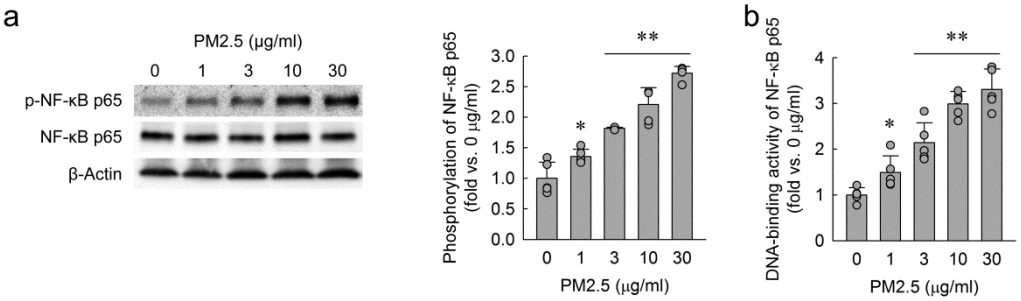

Figure 4. *PM2.5 activates NF-κB transcription factor in alveolar macrophages. MH-S cells were treated with 0, 1, 3, 10, or 30 μg/ml of PM2.5 for 24 h (n=5 cultures in each group). (a) NF-κB p65 phosphorylation (p-NF-κB p65) and its total expression (NF-κB p65) were examined using western blot analysis. Anti-β-actin antibody was used as a loading control. The intensities of these protein bands were quantified using the AzureSpot software (Azure Biosystems). (b) DNA-binding activities of NF-κB p65 were measured in the nuclear extracts of cells using the TransAM NF-κB p65 transcription factor assay kit (Active Motif). Data are shown as mean + s.d. *P<0.05 and **P<0.001 versus 0 μg/ml*

To determine whether PM2.5 exposure upregulates proinflammatory cytokines through NF-κB activation in alveolar macrophages, we treated with the two types of specific NF-κB inhibitors including Ro 106-9920 and PDTC in the MH-S cells. Treatments with the NF-κB inhibitors markedly reduced TNF-α, IL-1β, and IL-6 cytokines upregulated by PM2.5 exposure in a dose-dependent manner (*Fig. 5, a to c*). In particular, compared to IL-1β production, the TNF-α and IL-6 productions were more susceptible to a low concentration of these inhibitors (1 μM) (*Fig. 5, a to c*). These data suggest that PM2.5 upregulates proinflammatory cytokines through NF-κB activation in alveolar macrophages.

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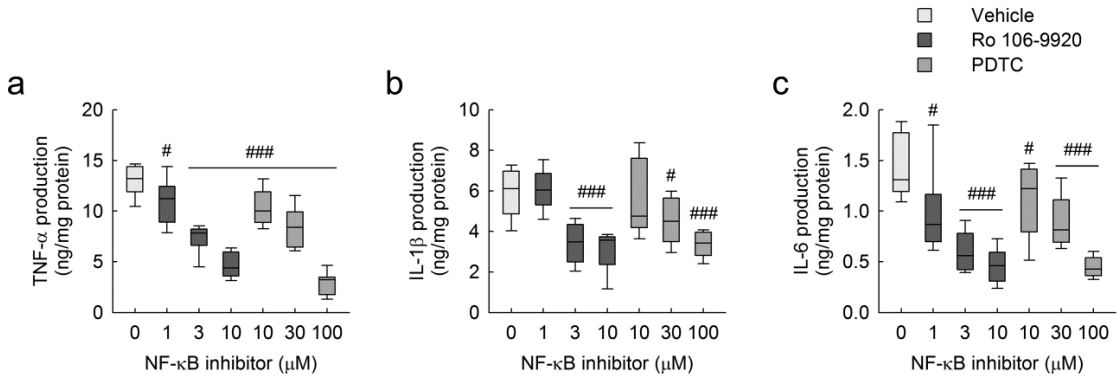

Figure 5. *NF-κB inhibition attenuates proinflammatory cytokine upregulation induced by PM2.5 in alveolar macrophages. MH-S cells were treated with 30 μg/ml of PM2.5 for 24 h. Some cells were treated with either Ro 106-9920 (1 to 10 μM), PDTC (10 to 100 μM), or vehicle (0.1% DMSO) immediately after PM2.5 administration (n=9 cultures in each group). Intracellular levels of cytokine TNF-α, IL-1β, and IL-6 were measured using multiplex immunoassay. In the box plots, whiskers represent the minimum and maximum; boxes represent the interquartile range between the first and third quartiles; and midlines represent the median. #P<0.05 and ##P<0.001 versus vehicle (0 μM NF-κB inhibitor)*

NF-κB inhibition suppresses PM2.5-induced M1 polarization in alveolar macrophages

In order to examine whether the NF-κB signaling pathway is involved in the PM2.5-induced M1 polarization in alveolar macrophages, we assessed the mRNA expression of M1/M2 marker genes at 24 h after PM2.5 administration in MH-S cells. Consistent with the patterns of proinflammatory cytokine production, the mRNA level of the M1 marker CD80 in the PM2.5-treated cells was significantly attenuated by treatments with the NF-κB inhibitors in a dose-dependent manner (*Fig. 6a*). However, the mRNA level of the M2 marker CD163 was not altered by the NF-κB inhibitions (*Fig. 6b*). Therefore, these data suggest that the NF-κB signaling pathway mediates the PM2.5-induced polarization of alveolar macrophages into M1 macrophages.

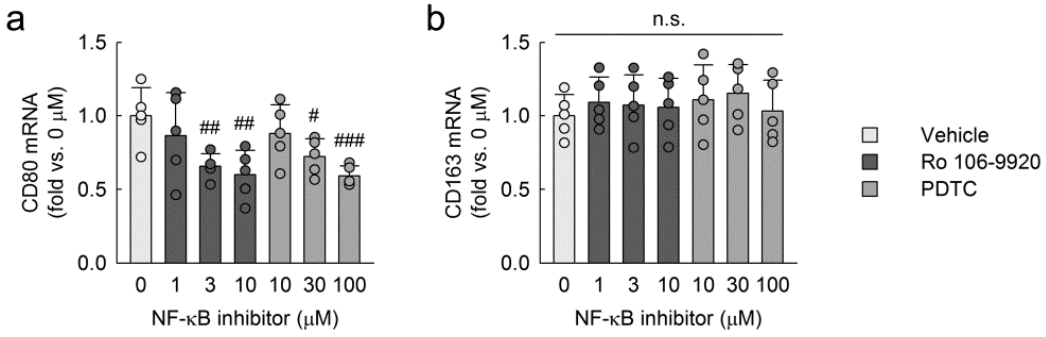

Figure 6. *NF-κB inhibition suppresses CD80 mRNA expression induced by PM2.5 in alveolar macrophages. MH-S cells were treated with 30 μg/ml of PM2.5 for 24 h. Some cells were treated with either Ro 106-9920 (1 to 10 μM), PDTC (10 to 100 μM), or vehicle (0.1% DMSO) immediately after PM2.5 administration (n=5 cultures in each group). Intracellular levels of CD80 and CD163 mRNA were measured using real-time quantitative RT-PCR (n=5 cultures in each group). Data are shown as mean + s.d. #P<0.05, ##P<0.01, and ###P<0.001 versus vehicle (0 μM NF-κB inhibitor); n.s., not significant*

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Discussion

Atmospheric PM pollution is a serious global issue. Long-term exposure to PM2.5 has been epidemiologically associated with respiratory mortality and disease (Kim et al., 2015b). Furthermore, PM2.5 concentration is implicated in the increasing number of incidences of upper respiratory tract infection, asthma, acute pulmonary disease (Cohen et al., 2005). Recently, many investigators have attempted to reveal associations of exposure to PM2.5 with respiratory inflammation through epidemiology and in vivo experiments. However, to protect against PM2.5-induced pulmonary inflammation, understanding of the underlying molecular mechanisms during the initiation and progression of inflammation is required. In this study, we found that in MH-S mouse alveolar macrophages, PM2.5 exposure upregulates proinflammatory cytokines and stimulates M1 macrophage polarization through the NF-κB signaling pathway. Our present data suggest that the NF-κB signaling plays a central role in proinflammatory macrophages during the development of pulmonary inflammation.

The alveolar macrophage has important microbicidal, phagocytic, and secretory functions and plays a distinctive role in lung immunity by initiating inflammatory and immune responses (Oberdörster et al., 1992). Furthermore, in order to sustain a well-organized host defense response, alveolar macrophages communicate with other cellular components of the innate immune system. Among the components, cytokines such as TNF-α, IL-1β, and IL-6 play a critical role in the pulmonary host defense. While inflammatory cytokines are beneficial for the host when produced in appropriate amounts, they are toxic when produced in a deregulated fashion. In this study, the TNF-a, IL-1β, and IL-6 cytokines produced by PM2.5 exposure in alveolar macrphages appeared to be similar to previously reported in vitro responses in either peritoneal macrophages or macrophage-like cell lines, which were not derived from the lung (Migliaccio et al., 2013; Pozzi et al., 2003; Soukup and Becker, 2001a). In addition, PM2.5 and its microenvironment directly affect the phenotype and function of primary mouse peritoneal macrophages; PM2.5 significantly enhances inflammatory M1 polarization and inhibits anti-inflammatory M2 polarization (Zhao et al., 2016). Interestingly, our data reveal that PM2.5 exposure in alveolar macrophages consistently stimulates M1 phenotypic polarization, as indicated by the high CD80 mRNA expression, while M2 phenotypic polarization is not affected by PM2.5 exposure as indicated by the non-altered CD163 mRNA expression. This possibility is supported by previous reports demonstrating that PM2.5 induces proinflammatory gene expression, while it does not contribute to anti-inflammation in other cells, including airway epithelial cells (Baulig et al., 2009), bronchial epithelial cells (Cachon et al., 2014), and peritoneal macrophages (Pozzi et al., 2003).

NF-κB transcription factors have been shown to transactivate a number of cytokines, chemokines, cell adhesion molecules, and receptors implicated in lung diseases (Yamamoto and Gaynor, 2001). PM2.5 and its components activate the NF-κB signaling pathway in lung and airway epithelial cells (Dagher et al., 2007; Marano et al., 2002). Consistent with this finding, our data demonstrates that PM2.5 exposure in alveolar macrophages induces NF-κB activation, leading to the upregulation of TNF-α, IL-1β, and IL-6 cytokines. Among the anti-inflammatory cytokines shown in our present data, IL-10 can be transactivated by NF-κB (Cao et al., 2006). The previous study showed that the homodimers of NF-κB transcription factor bind to the Il10 promoter in primary peritoneal macrophages, and loss of NF-κB subunit alters the IL-10 expression induced by lipopolysaccharide exposure (Cao et al., 2006). However, our

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present data showed that PM2.5-treated alveolar macrophages show NF-κB signaling activation, but do not alter the production of IL-10 protein. In addition to NF-κB, the expression of IL-10 can be regulated by other transcription factors including specific protein 1 (SP1), SP3, CCAAT/enhancer binding protein-β (C/EBPβ), interferon-regulatory factor 1 (IRF1), and signal transducer and activator of transcription 3 (STAT3) in macrophages (Saraiva and O'Garra, 2010), suggesting that the intracellular level of IL-10 production is independent of the NF-κB signaling. Therefore, the NF-κB activation induced by PM2.5 in alveolar macrophages specifically mediates the upregulation of proinflammatory cytokines as well as the polarization toward M1 phenotype. Previous reports have also supported that the NF-κB activation is essential for determining M1 polarization in macrophages (Barberi et al., 2015; Jang et al., 2013). Furthermore, the expression of the M1 marker CD80 is regulated and responsive to the NF-κB activation (Zhao et al., 1996).

Conclusion

The present results demonstrate that PM2.5 exposure induces the upregulation of proinflammatory cytokines TNF-α, IL-1β, and IL-6 through the NF-κB activation in alveolar macrophages and the polarization of alveolar macrophages to M1 phenotype. Furthermore, the results show that this mechanism might induce the inflammation cascade and pulmonary diseases. It is expected that inhibiting the NF-κB action will represent an effective therapeutic strategy to prevent or limit macrophage inflammation in pulmonary disease patients. In future studies using animal models of chronic obstructive pulmonary disease and acute lung injury, it needs to seek to determine whether PM2.5 induces pulmonary inflammation through the polarization of alveolar macrophages, and if so, whether PM2.5-induced inflammatory responses in alveolar macrophages is implicated in the activation of NF-κB. Finally, it needs to assess whether the genetic inhibition of NF-κB suppresses the development of pulmonary inflammation and diseases after exposure to PM2.5 in those animal models.

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