Review

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Airborne particles on pulmonary diseases

— Implication for immunodisruptors in the airway —

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Abstract

The concentration of airborne particulate matters (PM) in the environment affects daily hospital admissions for several pulmonary disorders such as bronchial asthma, acute and chronic bronchiolitis, and pneumonia. Especially, PM with a mass median aerodynamic diameter < or 2.5 μm ($PM_{2.5}$) is recognized to be more closely associated with respiratory effects and subsequent mortality than that with a mass median aerodynamic diameter < or 10 μm (PM_{10}). However, there is insufficient biological evidence underlying mechanisms to support these epidemiological investigations. In this review, we introduce the enhancing effects of PM, particularly, diesel exhaust particles as the main constituents of PM, on several pulmonary diseases, showing our *in vivo* evidence. Further, I also focus on the effects of exposure to nanoparticles/nano-materials, particles/materials less than 100 nm in mass median aerodynamic diameter, on the respiratory tract and disorders.

《**Key words**》 particulate matters, diesel exhaust particles, nanoparticles, acute lung inflammation, allergic asthma

Ⅰ. Introduction

Epidemiological studies have demonstrated a correlation between exposure to air pollutant particles at the concentrations currently found in major metropolitan areas and mortality and morbidity[1]. The concentration of particulate matter (PM) with a mass median aerodynamic diameter (a density-dependent unit of measure used to describe the diameter of particles) < or = 2.5 μm ($PM_{2.5}$) is more closely associated with both acute and chronic respiratory effects and consequent mortality than larger particles of < or = 10 μm (PM_{10})[2]. Moreover, one intriguing aspect of the epidemiologic data is that the health effects of $PM_{2.5}$ are primarily seen in subjects with predisposing factors, including pneumonia, asthma, chronic obstructive pulmonary disease, compromised immune systems, and an age over 65 years old (called as "sensitive subjects")[3]. Consistent with epidemiological studies, we have experimentally demonstrated that diesel exhaust particles (DEP), major contributors to environmental $PM_{2.5}$, exhibit respiratory toxicity in the presence or absence of predisposing factors *in vivo*[4～10].

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To date, additionally, nanoparticles, particles less than 100 nm in mass median aerodynamic diameter, have been found to be increasing in ambient air[11]. Recent measurements indicate that nanoparticle numbers in ambient air range from 2×10^4 to $2\times10^5/cm^3$, with mass concentrations of more than $50\,\mu g/m^3$ near major highways[12,13]. Further, nanotechnology is now advancing at an incredible pace, such that it is has created an alternative industrial revolution over the past few years[14]. Consistent with this, the use of engineered nanomaterials has been rapidly increasing in commercial applications. As these materials have become more widespread, many questions have arisen regarding the effects they may have on the environment as alternative inhalable toxicants. Due to their size, nanoparticles/nanomaterials have been implicated in cardiopulmonary system effects[15]. Compared to larger particles, nanoparticles have a higher deposition rate in the peripherial lung, they can cross the pulmonary epithelium and reach the interstitium[16], and furthermore, may be systemically distributed in the bloodstream[17]. Nanoparticles have an enhanced capacity to produce reactive oxygen species, and, consequently, exhibit widespread toxicity[18~20]. In consistent with these *in vitro* and *in vivo* reports, nanoparticle exposure also reportedly influences cardiopulmonary systems in the presence or absence of predisposing diseases in human studies[21,22].

Ⅱ. Effects of airborne particles on acute lung inflammation induced by bacterial endotoxin

A glycolipid of gram-negative bacteria, known as endotoxin or lipopolysaccharide (LPS), stimulates host cells to elicit various immune reactions[23]. In animal models, the intratracheal administration of LPS causes lung cytokine production, neutrophil recruitment, and lung injury[24]. LPS is found in the bronchoalveolar lavage (BAL) fluid of patients with pneumonia[25] and acute respiratory distress syndrome[26], which sometimes results in a fatal outcome. In addition, LPS is a significant constituent of many air pollutant particles and has, accordingly, been implicated in PM effects[27]. In accordance with the close links among LPS, lung inflammation, and PM, we previously demonstrated that pulmonary exposure to DEP and their components facilitates lung inflammation induced by LPS and subsequent systemic inflammation with coagulatory impairment[8,9,28].

In our previous experiment, DEP were extracted with dichloromethane (CH_2Cl_2) and the extracts were prepared as DEP derived organic chemicals (DEP-OC: Ref 28). On the other hand, residual particles of DEP were prepared as washed DEP. Then, male ICR mice were divided into six experimental groups, which received vehicle (control), washed DEP, DEP-OC, LPS, washed DEP + LPS, or DEP-OC + LPS. All were inoculated intratracheally. As a result, histopathologically, the lung specimens showed that LPS induced the moderate infiltration of neutrophils. The combined exposure to washed DEP and LPS markedly enhanced neutrophil sequestration, interstitial edema, and alveolar hemorrhage as compared with LPS exposure alone. The histological changes caused by DEP-OC + LPS exposure were less prominent than those by washed DEP + LPS exposure. Further, LPS exposure significantly increased the protein concentrations of interleukin (IL)-1β, macrophage inflammatory protein (MIP)-1α, macrophage chemoattractant protein (MCP)-1, and keratinocyte-derived chemoattractant (KC) as

compared with vehicle exposure. Combined exposure to washed DEP and LPS resulted in further, significant increases as compared with LPS exposure alone. The results of these proinflammatory molecules were concomitant with those in neutrophilic inflammation with pulmonary edema. DEP-OC + LPS exposure did not increase the concentrations of these proinflammatory molecules compared with LPS exposure. Also, exposure to LPS significantly increased gene expression for IL-1β and MIP-1α compared with vehicle exposure. Exposure to washed DEP + LPS further increased the mRNA expression for these proinflammatory molecules as compared to LPS exposure, whereas DEP-OC + LPS exposure did not. Moreover, LPS exposure elevated the mRNA expression for toll-like receptor (TLR)-2 as compared with vehicle exposure. The expression of TLR-2 was more prominent in the DEP-OC + LPS and washed DEP + LPS groups than in the LPS group. The expression was most prominent in the washed DEP + LPS group. Exposure to DEP-OC, washed DEP, LPS, or DEP-OC + LPS slightly increased TLR4 expression compared with vehicle exposure. A larger rise in TLR4 expression was induced in the washed DEP + LPS group than in the LPS group[28]. Furthermore in another experiment, the degree of systemic inflammation with coagulatory disturbance accompanied by LPS-related lung injury, a causal risk factor for cardiac attack[29], was evidenced to show a similar trend to the lung inflammatory response as described above[9].

We subsequently examined the effects of pulmonary exposure to nanoparticles (using an intratracheal instillation technique) on lung inflammation related to LPS in mice. Vehicle, two sizes (14 and 56 nm) of carbon black nanoparticles, LPS, or LPS + nanoparticles was administered intratracheally, and parameters for lung inflammation and coagulation were evaluated. Nanoparticles alone induced slight lung inflammation and significant pulmonary edema as compared with the vehicle. Fourteen-nanometer nanoparticles intensively aggravated LPS-elicited lung inflammation and pulmonary edema, whereas 56 nm nanoparticles did not show apparent effects, which was concomitant with the enhanced lung expression of IL-1β, MIP-1α, MCP-1, MIP-2, and KC regarding the overall trend[30]. Immunoreactivity for 8-hydroxyguanosine (8-OHdG), a proper marker for oxidative stress, was more intense in the lung of the LPS + 14 nm nanoparticle group than that in the LPS group. The circulatory fibrinogen level was higher in the LPS + 14 nm nanoparticle group than in the LPS group. Taken together, nanoparticles can aggravate lung inflammation related to bacterial endotoxin, which is more prominent with smaller particles. The enhancement may be mediated, at least partly, via the increased local expression of proinflammatory cytokines and oxidative stress. Furthermore, nanoparticles can promote coagulatory disturbance accompanied by lung inflammation[30]. Thereafter, we found that latex nanoparticles[31], TiO2 nanoparticles[32], and carbon nanotubes[33] have similar effects on the lung pathophysiology.

Taken together, these studies suggest that airborne particles such as DEP and several nanoparticles/nanomaterials can synergistically exacerbate infectious lung inflammation (Fig.) with partially systemic inflammation with coagulatory impairment. Furthermore, the particulate components and sizes of such particles may be responsible for these aggravations.

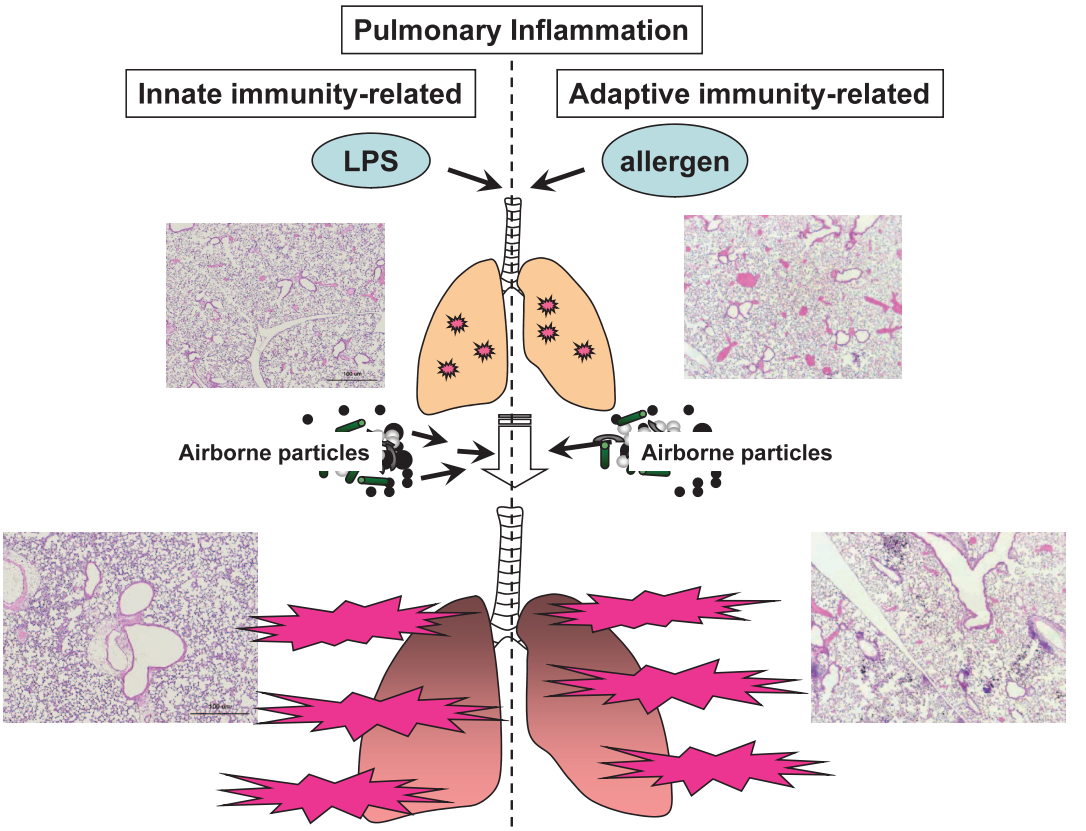

Fig. Proposed schema for facilitating effects of airborne particles on predisposing lung diseases.

Ⅲ. Effects of airborne particles on allergic asthma

Bronchial asthma has been recognized as chronic airway inflammation that is characterized by an increase in the number of activated lymphocytes and eosinophils.

DEP or organic chemicals in DEP are known to aggravate allergic responses. Organic chemicals in DEP enhance the production of antigen-specific IgE *in vitro*[34,35] and *in vivo*[36]. Pyrene, a major organic compound in DEP, affects the production of IL-4 *in vitro*[37]. The intranasal injection of organic chemicals in DEP into mice reportedly induced the recruitment of eosinophils and increased the protein level of IL-5 in BAL fluid[38]. In contrast, there are few reports on the effects of DEP after the extraction of organic chemicals on allergic airway inflammation. We previously reported that intratracheal challenge of DEP with ovalbumin (OVA: an established model allergen) enhances OVA-related eosinophilic inflammation, mucus secretion, cytokine expression, and Ig responses[7].

Also, we investigated the effects of DEP components on allergic airway inflammation. Male ICR mice were divided into eight experimental groups, which received: vehicle (control), washed DEP, DEP-OC, whole DEP, OVA, washed DEP + OVA, DEP-OC + OVA, or whole DEP + OVA. All groups received OVA or vehicle every two weeks for six weeks, and DEP components or vehicle once a week for six weeks. All were inoculated intratracheally.

The lung specimens showed that the infiltration of eosinophils and neutrophils (polymorphonuclear leukocytes: PMNs), mononuclear cells, and goblet cell proliferation were slight in the DEP-OC, washed DEP, whole DEP, and OVA groups. Exposure to OVA + DEP-OC or OVA + whole DEP induced a more prominent infiltration by PMNs than that to OVA alone. The infiltration was most prominent on exposure to OVA + whole DEP. Exposure to DEP components + OVA enhanced the infiltration of mononuclear cells around the airways as compared with vehicle exposure. Exposure to DEP-OC + OVA and whole DEP + OVA led to marked increases in the number of mononuclear cells compared with OVA exposure alone. The expression of Th1 and Th2 cytokines in the lung tissue supernatants demonstrated that combined pulmonary exposure to DEP-OC + OVA significantly increased the protein level of IL-5 compared with OVA exposure alone. Combined exposure to whole DEP + OVA resulted in a further, significant increase in IL-5. Combined exposure to whole DEP + OVA resulted in a marked elevation of IL-13. The expression of interferon (IFN)-γ, a Th1-type cytokine, was significantly greater in the washed DEP + OVA group than in the OVA group. The protein level of chemokine (c-c motif) ligand 11 (CCL11) in the OVA group was significantly higher than in the vehicle group. The DEP-OC + OVA group showed a further, significant increase in CCL11 as compared with the OVA group. Furthermore, combined exposure to whole DEP + OVA significantly enhanced the expression of CCL11. The expression of MIP-1α was significantly higher in the whole DEP group than in the vehicle group. Exposure to whole DEP + OVA lead to a further, significant increase in MIP-1 α as compared with exposure to vehicle, OVA, washed DEP + OVA, or DEP-OC + OVA. Further, exposure to DEP-OC significantly increased the production of OVA-specific IgG1 as compared with vehicle exposure alone. Combined exposure to whole DEP + OVA markedly enhanced OVA-specific IgG1[39].

Carbon black has been demonstrated to enhance the proliferation of antibody-forming cells and both IgE and IgG levels[40, 41]. Ultrafine particles (PM and carbon black) reportedly aggravate allergic airway inflammation *in vivo*[42, 43]. However, no studies have described the size of particles they used. We investigated the effects of nanoparticles with a diameter of 14 or 56 nm on allergic airway inflammation. ICR mice were divided into six experimental groups. Vehicle, two sizes of carbon nanoparticles, OVA, and OVA + nanoparticles were administered intratracheally. The cellular profile of BAL fluid, lung histology, expression of cytokines, chemokines, 8-OHdG, and immunoglobulin production were studied. Nanoparticles with a diameter of 14 or 56 nm aggravated allergic airway inflammation, characterized by the infiltration of eosinophils, neutrophils, and mononuclear cells, and by an increase in the number of goblet cells in the bronchial epithelium. Nanoparticles with allergen increased protein levels of IL-5, IL-6, and IL-13, CCL11, MCP-1, and regulated on activation and normal T cells expressed and secreted (RANTES) in the lung as compared with antigen alone. The formation of 8-OHdG was moderately induced by nanoparticles or allergen alone, and was markedly enhanced by allergen plus nanoparticles as compared with nanoparticles or allergen alone. The aggravation was more prominent with 14- compared to 56 nm nanoparticles in the context of the overall trend. Particles with a diameter of 14 nm exhibited adjuvant activity for total IgE and al-

lergen-specific IgG and IgE. Nanoparticles can aggravate allergic airway inflammation and immunoglobulin production, which is more prominent with smaller particles. The enhancement may be mediated, at least partly, by the increased local expression of IL-5 and CCL11, and also by the modulated expression of IL-13, RANTES, MCP-1, and IL-6[44]. Interestingly, lung physiology test yielded slightly different results in which 56-nm carbon nanoparticles predominantly aggravated airway hyperresponsiveness induced by OVA exposure[45]. Furthermore, we additionally demonstrated that carbon nanotubes promote allergic airway inflammation in mice[46, 47].

Taken together, these studies suggest that environmental particles such as DEP and several types of nanoparticle/nanomaterial can synergistically exacerbate allergic airway inflammation (Fig.). Furthermore, organic chemical components in such particles may be responsible for these aggravations. Also, nano-leveled particles exacerbate the pathology, and this exacerbation may be worse in the presence of smaller compared to larger particles.

Ⅳ. Model's relevance to the actual situation and future perspectives

In reality, however, we inhale suspended DEP and/or nanoparticles in ambient air, but do not intratracheally receive DEP and/or nanoparticles suspensions in aliquots. Nevertheless, assessment of the impact of inhalation exposure to diesel engine-derived nanoparticles, a more realistic exposure, on the lung inflammation model has never been conducted. Furthermore, as far as we know, no study has examined the dose-dependent effects of inhaled nanoparticles on predisposed subjects. In our previous study, nanoparticle inhalation exaggerated lung inflammation induced by LPS. Enhancement was the most prominent with a particulate concentration of approximately 169, followed by 36, and then 15 $\mu g/m^3$, suggesting that the particulate concentration is important for enhancement. Moreover, it is surprising that the concentrations that showed prominent enhancing effects in the present study (169 and 36 $\mu g/m^3$) are comparable to or not much higher than those previously reported to be measured in places near major highways which convey large numbers of diesel-engine automobiles (50 $\mu g/m^3$ as $PM_{2.5}$ concentration; Zhu et al.,[13]; Timonen et al.,[12]). Thus, it is possible that inhaled nanoparticles also exacerbated lung inflammation induced by LPS in a concentration-dependent manner. Nevertheless, the effects of nanoparticles generated by diesel engines instruments on several other cardiopulmonary conditions, especially in sensitive populations, should be elucidated in the future.

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