Facilitating Effects of Nanoparticles/Materials on Sensitive Immune-Related Lung Disorders

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1. 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 the particle) ≤ 2.5 μm (PM₂.₅) is more closely associated with both acute and chronic respiratory effects and subsequent mortality than larger particles of ≤ 10 μm (PM₁₀) [2]. In addition, one intriguing aspect of the epidemiologic data is that health effects of PM₂.₅ are primarily seen in subjects with predisposing factors, including pneumonia, asthma, chronic obstructive pulmonary disease, compromised immune systems, atherosclerosis, age over 65 years old, and maybe depressive states [3–6]. Partially consistent with the epidemiological studies, we and others have experimentally demonstrated that diesel exhaust particles (DEP), major contributors to environmental PMₓ₂.₅ in urban areas, exhibit respiratory toxicity with or without predisposing pathologies including allergic asthma, pulmonary emphysema, and acute renal failure in vivo [7–15].

To date, nanoparticles, particles less than 0.1 μm in mass median aerodynamic diameter, have been shown to be increasing in ambient air [16]. Recent measurements indicate that nanoparticle numbers in ambient air range from 2 × 1₀⁴/cm³ to 2 × 1₀⁵/cm³, with mass concentrations of more than 50 μg/m³ near major highways [17, 18]. Also, nanoparticles have been implicated in cardiopulmonary system effects [19]. Furthermore, compared to larger particles, nanoparticles have a higher deposition rate in the peripheral lung, can cross the pulmonary epithelium, reach the interstitium [20], and may thus be systemically distributed in the bloodstream [21]. Nanoparticles have an enhanced capacity to produce reactive oxygen species, and consequently have a widespread toxicity [22–24].
Further, nanotechnology is now advancing at such an incredible pace that it has created an alternative industrial revolution over the past few years [25]. 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 adverse effects they may have on the environment as alternative inhalable toxicants. Due to their sizes, characteristics, and/or existing pattern, nanoparticles/materials have been implicated in cardiopulmonary system effects [19]. Compared to larger particles, nanoparticles have a higher deposition rate in the peripheral lung, can cross the pulmonary epithelium reach the interstitium [20], and, furthermore, may be systemically distributed in the bloodstream [21]. Furthermore, nanoparticles have an enhanced capacity to produce reactive oxygen species, and, consequently, have a widespread toxicity [22–24]. Nanoparticle exposure also reportedly influences cardiopulmonary systems in the presence or absence of predisposing diseases in human studies [26, 27]. However, biological evidence concerning the promoting effects of nanoparticles/materials on predisposing subjects has been less studied. Besides their toxic effects on health, therefore, it should be experimentally ascertained whether they also aggravate preexisting pathological conditions, and their underlying mechanisms should be resolved. In this paper, therefore, we will discuss the impact of nanoparticles/materials as immunological enhancers.

2. Effects of Nanoparticles on Acute Lung Inflammation Induced by Bacterial Endotoxin

A glycolipid of gram-negative bacteria, known as endotoxin or lipopolysaccharide (LPS), stimulates host cells via innate immunity [28]. In animal models, intratracheal administration of LPS causes lung cytokine expression, neutrophil recruitment, and lung injury [29]. LPS is found in bronchoalveolar lavage (BAL) fluid of patients with pneumonia [30] and acute respiratory distress syndrome [31], 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 the adverse effects of PM [32]. In accordance with the close links among LPS, lung inflammation (injury), and PM, we have previously shown that intratracheal administration of DEPs and their components facilitates lung inflammation induced by LPS [13, 33] and subsequent systemic inflammation with coagulatory impairment [14].

We next examined the effects of pulmonary exposure to nanoparticles 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 of lung inflammation and coagulation were evaluated. Nanoparticles alone induced slight lung inflammation and significant pulmonary edema as compared with the vehicle. Fourteen-nm nanoparticles intensively aggravated LPS-elicited lung inflammation and pulmonary edema, which was concomitant with the enhanced lung expression of interleukin (IL)-1β, macrophage inflammatory protein (MIP)-1α, macrophage chemoattractant protein (MCP)-1, MCP-2, and keratinocyte chemoattractant (KC) in overall trend, whereas 56-nm nanoparticles did not show apparent effects. Immunoreactivity for 8-hydroxyguanosine (8-OHdG), a proper marker for oxidative stress, was more intense in the lung from the LPS + 14-nm nanoparticle group than that from the LPS group. The circulatory fibrinogen level was higher in the LPS + 14-nm nanoparticle group than that in the LPS group. Taken together, nanoparticles can aggravate lung inflammation related to bacterial endotoxin, which is more prominent with smaller particles. The enhancing effect may be mediated, at least partly, via the increased local expression of proinflammatory cytokines and via the oxidative stress. Furthermore, nanoparticles can promote coagulatory disturbance accompanied by lung inflammation [34].

Furthermore, we examined the adverse effects of nanomaterials on this pathological model. In brief, ICR male mice were divided into 8 experimental groups that intratracheally received vehicle, three sizes (15, 50, 100 nm) of TiO2 nanomaterials, LPS, or LPS plus nanomaterials. Twenty-four hours after the treatment, both nanomaterials exacerbated the lung inflammation and edema elicited by LPS, with an overall trend of amplified lung expressions of cytokines such as IL-1β, MCP-1, and KC. LPS plus nanomaterials, especially with size less than 50 nm, elevated circulatory levels of fibrinogen, IL-1β, MCP-1, KC, and von Willebrand factor as compared with LPS alone. The enhancement tended overall to be greater with the smaller nanomaterials than that with the larger ones. cDNA microarray analyses revealed that gene expression pattern was not different between the LPS group and the LPS + nanomaterial groups. These results suggest that nanomaterials exacerbate lung inflammation related to LPS with systemic inflammation and coagulatory impairment, and the exacerbation is more prominent with smaller nanomaterials than that with larger ones (35) and unpublished data). Additionally, we demonstrated that latex nanoparticles [36] and carbon nanotubes [37] have similar adverse effects on the lung pathophysiology.

Our next study was conducted to determine whether inhaled exposure to diesel engine-derived nanoparticles also exacerbates the model. ICR mice were exposed for 5 hours to clean air or diesel engine-derived nanoparticles at a concentration of 15, 36, or 169 μg/m3 after intratracheal challenge with LPS or vehicle, and were sacrificed for evaluation 24 hours after the inhaled exposure. Exposure to nanoparticles alone did not elicit lung inflammation. Nanoparticle inhalation exaggerated LPS-elicited inflammatory cell recruitment in the BAL fluid and lung parenchyma as compared with clean air inhalation in a concentration-dependent manner. Lung homogenates derived from the LPS + nanoparticle groups tended to have an increased tumor necrosis factor-α level and chemotaxis activity for polymorphonuclear leukocytes as compared with those from the LPS group or the corresponding nanoparticle groups. Nanoparticle inhalation did not significantly increase the lung expression of proinflammatory cytokines or influence
systemic inflammation. Isolated alveolar macrophages from nanoparticle-exposed mice showed a greater production of IL-1β and KC stimulated with ex vivo LPS challenge than those from clean air-exposed mice although the differences did not reach significance. These results suggest that acute exposure to diesel-nanoparticles exacerbates lung inflammation induced by LPS [38]. In sum, nanoparticle/material exposure exacerbates acute lung inflammation related to bacterial endotoxin (Figure 1).

3. Effects of Nanoparticles on Allergic Airway Inflammation

Bronchial asthma has been recognized as chronic airway inflammation with hyperresponsiveness that is characterized by an increase in the number of activated lymphocytes and eosinophils [39]. A number of studies have shown that various particles including carbon black can enhance allergic sensitization [40–42], which is referred to as “adjuvant effect” as well. Carbon black has been demonstrated to enhance the proliferation of antibody-forming cells and both IgE and IgG levels [43, 44]. Ultrafine particles (PM and carbon black) reportedly exaggerate allergic airway inflammation in vivo [45, 46]. However, all studies have failed to pay attention to the size of particles. Therefore, no research has addressed the size effects of particles or nanoparticles on airway biology in the presence or absence of allergen in vivo. Given the hypothesis, we investigated the effects of carbon black nanoparticles with a diameter of 14 nm or 56 nm on allergen-related airway inflammation. ICR mice were divided into six experimental groups. Vehicle, two sizes of carbon nanoparticles, ovalbumin (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 nm or 56 nm aggravated antigen-related 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 antigen increased protein levels of IL-5, IL-6, IL-13, eotaxin, MCP-1, and regulated upon 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 nm nanoparticles than that with 56-nm particles in terms of the overall trend. Particles with a diameter of 14 nm exhibited an adjuvant activity for total IgE and antigen-specific IgG and IgE. Nanoparticles can aggravate allergen-related airway inflammation and immunoglobulin production, which become more prominent with smaller particles. The enhancement may be mediated, at least partly, by the increased local expression of IL-5 and eotaxin, and also by the modulated expression of IL-13, RANTES, MCP-1, and IL-6 [47]. Consistent with our study, de Haar and colleagues have previously shown that nanoparticles (14 and 29 nm) potently facilitate allergic airway inflammation as compared with fine particles (250 and 260 nm) [48].

In ongoing reports, nanoparticles alone or OVA alone moderately enhanced cholinergic airway reactivity, as assessed by total respiratory system resistance (R) and Newtonian resistance (Rn). All the parameters of lung responsiveness, such as R, compliance, elastance, Rn, tissue damping, and tissue elastance, were worse in the OVA + nanoparticle groups than those in the vehicle group, the corresponding nanoparticle groups, or the OVA group. The lung mRNA level for Muc5ac was significantly higher in the OVA group than that in the vehicle group, and further increased in the OVA + nanoparticle groups than that in the OVA or nanoparticle groups. These data suggest that carbon nanoparticles can enhance lung hyperresponsiveness, especially in the presence of allergen. The effects may be mediated, at least partly, through the enhanced lung expression of Muc5ac [49].

Furthermore, we recently demonstrated that (single-walled and multiwalled) carbon nanotubes promote allergic airway inflammation in mice, which may be partly through enhanced oxidative stress in the airway and the inappropriate activation of antigen-presenting cells including dendritic cells (in vitro) [50, 51]. In addition, other groups have reported the similar impacts of nanomaterials (carbon nanotubes, TiO2, and on gold) us as on animal allergic asthma models [48, 52–54]. Moreover, as for cellular contribution, we and others have claimed that antigen-presenting cells such as dendritic cells are important target cell populations for the adjuvant activity of nanoparticles/materials [55–57]. Taken together, nanoparticle/material exposure can exacerbate allergic asthma (Figure 1).

4. Considerations for Future Directions

4.1. Risk Factors Regarding Nanoparticles. One important point to be taken into consideration in these studies is the
surface characteristics and numbers of nanoparticles used. Our results indicate that nanoparticles, particularly smaller ones (14 nm in diameter), can aggravate lung inflammation related to LPS and allergic airway inflammation when the weights of particles are equal. On the other hand, the surface area of the 14-nm nanoparticles was 6.7 fold larger than that of 56-nm nanoparticles (300 m²/g versus 45 m²/g, resp.). The surface area of particles exposed to is reportedly correlated with lung inflammation [37]. Alternatively, our studies have demonstrated not only the size effects of nanoparticles on lung inflammation, but also the effects of their surface area and/or their numbers on the inflammation.

Unfortunately, we could not examine the effects of nanoparticles with the same particle number in these studies. The number of smaller nanoparticles is greater than that of larger nanoparticles when the particles comprise the same weight.

4.2. Possibility of Migration and Influence of Nanoparticles Exposed to the Airway into Systemic Circulation. It also remains to be argued whether nano-level particles/materials delivered through the airway enter the systemic circulation and cause serve adverse effects such as systemic inflammation, and thrombus formation. Nanoparticles are reportedly able to penetrate deeply into the respiratory tract and can even pass the lung to reach systemic circulation [58, 59]. Nemmar et al. have previously demonstrated that nanoparticles can migrate into the circulation [59]. In our study, the LPS + nanoparticle groups, specifically the LPS + 14-nm nanoparticle group, showed significantly higher fibrinogen levels when compared to the LPS group. Additionally, although not significant, the enhanced activity of vWF induced by LPS was further increased by its combination with 14-nm nanoparticles [34]. These findings suggest that smaller nanoparticles can facilitate coagulatory disturbance accompanied by lung inflammation. The enhancing effects of 14-nm nanoparticles on LPS-elicited pulmonary edema further support this concept. Interestingly, exposure to nanoparticles alone did not induce significant fibrinogen production/release nor did it activate vWF. It might be hypothesized that endothelial-epithelial damage induced by LPS and subsequent infiltrating effector leukocytes allow a large amount of smaller nanoparticles to pass easily into the circulation, resulting in synergistic effects on hemostasis including coagulatory disturbance. On the other hand, exposure to environmental particles reportedly generates local and systemic oxidative stress, which, in turn, induces/enhances inflammation and blood coagulation [58]. Further, Nemmar and colleagues have demonstrated that nanoparticles instilled intratracheally rapidly diffuse from the lung into the systemic circulation in vivo [59]. Therefore, it is also possible that intratracheally instilled nanoparticles enter the circulation by themselves and contribute to a high susceptibility to LPS-elicited systemic inflammation and coagulatory disturbance. Future studies are needed to confirm the penetration and address the above-mentioned hypothesis.

4.3. Model's Relevance to the Actual Situation. In reality, we inhale suspended particulate matters in ambient air, but do not intratracheally receive them in aliquot. Nevertheless, the impacts of inhalation exposure to these particles/materials, the more realistic exposure, on this lung inflammation model had less been conducted by us and others. In our previous study, nanoparticle-rich diesel exhaust inhalation exaggerated lung inflammation induced by LPS [38]. Nonetheless, we have not completed/examined the effects of the inhalation on other disease models. In future, therefore, more realistic research considering the effects of the mode of nanoparticles/materials administration (instillation versus aerosolization, droplets versus powder, etc.) on the in vivo response would be very valuable to toxicologists, environmental scientists, and immunologists.

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References


