

# Toxicological Biology of Particulate Matters on Allergic Pulmonary Inflammation

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## ABSTRACT

Although the adverse health effects of Particulate Matters (**PM**) have been proposed and are being clarified, their facilitating effects on preexisting pathological conditions (pathological conditions) have not been fully identified. On the other hand, there exist hypersensitive subjects against PM. In this review, we provide insights into the immunotoxicity of PM as an aggravating factor in hyper susceptible subjects, especially those with allergic pulmonary diseases using our *in vivo* experimental models. In brief, we examined the effects of Diesel Exhaust Particles (**DEP**), main constituents in PM, on allergic asthma *in vivo*, and showed that repetitive pulmonary exposure to DEP has promoting effects on allergic airway inflammation, including adjuvanticity on Th2-milieu. Further, we propose a causal toxicological machinery regarding the adverse impacts, i.e., via inappropriate activation of antigen-presenting cells such as dendritic cells.

**Keywords:** Diesels exhaust particles; Allergic pulmonary inflammation; Antigen-presenting cells; Dendritic cell; Th response

## OVERVIEW

Motor vehicle emissions are a major source of airborne pollutants [1]. The combustion of fossil fuels produces a number of unhealthy substances, such as carbon monoxide, nitrogen oxides, benzene, sulfur dioxides, and Particulate Matters (**PM**). The largest single source of airborne PM from vehicles is derived from diesel exhaust. Advances in technology have resulted in diesel engines that produce less nitrogen oxide and particulate mass. However, diesel engines continue to emit higher emissions of gaseous substances and up to 100 times more particles compared with gasoline engines. In addition, with the high cost of fuel in many countries, use of diesel engines has increased due to their superior energy efficacy and endurance. Diesel fuel combustion results in the production of Diesel Exhaust Particles (**DEP**), as well as gaseous compounds. DEP consist of an elemental carbon core with a large surface area to which hundreds of chemicals and metals are attached. The majority of DEP are classified as fine (2.5-0.1 $\mu$ m) or nano (<0.1 $\mu$ m) particles. It is accepted that DEP possess various health toxicity from mutagenesis to proinflammation. In this review, we provide insights into the immunotoxicity of DEP as an aggravating factor in hyper susceptible subjects, in particular, those with allergic pulmonary diseases using our *in vivo* experimental model.

## OBSERVATIONAL STUDIES ON ADVERSE HEALTH EFFECTS OF DIESEL EXHAUST PARTICLES

Previous epidemiological studies have indicated that exposure to ambient PM is linked to increases in mortality and morbidity related to cardiopulmonary diseases [2,3]. Reportedly, increased rates of myocardial infarction and hospitalization for cardiac events have been associated with higher air pollution concentrations [4-6]. In addition, numerous studies have shown associations between increased symptoms of cough, bronchitis, asthma, and chronic obstructive pulmonary disease and increased air pollutant levels [7-9]. Further, a study has shown that PM with a diameter less than 2.5 $\mu$ m (PM 2.5) is more closely associated with both acute and chronic respiratory effects including bronchial asthma and subsequent mortality than PM with a diameter less than 10  $\mu$ m (**PM10**) [10]. On the other hand, some studies implicated the association between development of several types of cancer (lung, bladder, leukemia) and degree of air pollution including diesel exhaust (particles) [11-14].

## DEP AS IMMUNE DISRUPTORS

In general, experimentally, DEP are implicated to act as immune disruptors. For instances, DEP and/or their components enhance collagen-induced arthritis in mice, which is concomitant with amplified production of anti-collagen antibodies and secretion of Interleukin (**IL**)-2 and Interferon (**IFN**)- $\gamma$  [15] and induce/elevate lupus-related specific auto antibodies in mice [16]. (manuscript in preparation) In addition, the previous studies on intranasal administration have reported that DEP with antigen demonstrate adjuvant activity for IgE production in mice by passive cutaneous

anaphylaxis assay [17] and that those without antigen induce nasal nonspecific IgE response in humans [18,19]. These previous reports strongly suggest that DEP can disrupt immune system maybe toward inappropriate stimulation/activation of polyclonal immunoglobulin production.

## ADJUVANT EFFECTS OF DEP ON ALLERGIC ASTHMA

Also, some data imply that PM is a risk factor for the development/exacerbation of allergic diseases' phenotype including bronchial asthma, allergic rhinitis, and atopic dermatitis [20,21]. In particular, during PM pollution episodes, asthmatic subjects reportedly demonstrate increased respiratory symptoms, bronchoconstriction, medication use, bronchial hyper reactivity, and emergency care visits [22,23]. Compatible with the above mentioned epidemiological investigation, we and others have experimentally demonstrated that exposure to DEP, representative constituents in PM 2.5; exacerbate experimental asthma [24-28]. It has also been shown that airway exposure to DEP enhanced lung expression of Th cytokines (IL-4, IL-5, and/or IFN- $\gamma$ ) in the presence of antigen [25,27,28] indicating that DEP facilitates antigen-related airway inflammation, at least partly, through the enhanced Th response in the lung. Furthermore, our consecutive research have shown that repetitive pulmonary exposure to DEP *in vivo*, if combined with allergen, amplifies *ex vivo* allergen-specific Th2 response in the lung compared to that to allergen alone, characterized by high levels of IL-4 and IL-5, suggesting that in asthmatic subjects, DEP promote Th2-prone milieu in the lung, which additively/synergistically augment asthma pathophysiology *in vivo* [29]. As well, we have demonstrated that *in vivo* DEP exposure amplified extrathoracic CD4 polarization in asthmatic subjects [30].

## DEP ON DC-T CELL AXIS

The role of Antigen-Presenting Cells (**APC**), including dendritic cells, macrophages, and B cells, in the pathogenesis of allergic diseases has been relatively well defined. When antigens enter the airways, APC in the airways capture antigens and migrate to secondary lymphoid tissues such as draining lymph nodes [31]. There, antigen-specific T cells differentiate into Th effector cells or regulatory T cells via an interaction with APC [32]. Indeed, pathological activation of APC reportedly enhances antigen-related airway inflammation *in vivo* [33,34]. Among APC, Dendritic Cells (**DC**) are recognized to be professional cell populations having potent antigen-presenting properties [31,32, 35,36]. Several studies have identified a critical role for DC in antigen-related airway inflammation [37,38]. Indeed, *in vitro* data demonstrated that DEP exposure drives DC maturation [39,40] via epithelial cell-derived mediators [41, 42] and that DEP-exposed DC induces a Th2 polarization [39,43,44].

On the other hand, proallergic effects of DEP on T lymphocytes primed with *in vivo* antigen challenge remain obscure, although previous *in vivo* studies have demonstrated that DEP amplify the lung expression of Th cytokines [25,27,45]. Indeed, it has been previously reported that one of components of DEP, pyrene, induces a Th milieu in both human and murine T cells *in vitro* [46].

In our previous study, the numbers of cells expressing MHC class II and/or co-stimulatory molecules (CD80 and CD86) in the lung were greater in the DEP + OVA group than in the other groups [40]. Furthermore, the numbers of DC, macrophages, and B cells bearing MHC class II in the lung were also increased by the administration of DEP to antigen-sensitized mice. It can be proposed that the enhanced expression of these APC-related molecules and increased pulmonary numbers of APC in the presence of antigen induced by DEP can, at least in part, play a role in the aggravating effects of DEP on the allergic response in the airway as reported previously [27,28]. Otherwise, the current data may lead to the scenario that DEP can synergistically increase the number of APC recruited into the lung in asthmatic mice, and in turn, can amplify functional activation of DC, which confer synergism for the enhancement of asthma pathophysiology.

Our previous study showed that DEP enhanced the response/pathway as demonstrated by the allogenic Mixed Lymphocyte Reaction (**MLR**) and in particular, antigen-specific syngeneic lymphocyte reaction [40]. Also, we previously demonstrated that pulmonary exposure to DEP and their components amplified lung expressions of Th cytokines *in vivo* [27,28]. In our recent study, when splenic mononuclear cells from the DEP + OVA group were stimulated with OVA, we found that they produced a greater amount of Th cytokines than those from the other groups [41]. Accordingly, the *ex vivo* results suggest that the enhancing effects of DEP on allergic asthma can be explained, at least in part, by skewing T cells toward a Th phenotype, especially during antigen priming. Furthermore, our *in vitro* and *in vivo* studies suggest that this Th-skewed susceptibility found in DEP plus OVA - primed lymphocytes could be mediated, at least in part, through amplified APC (**DC**) activity.

## RESEARCHES ABOUT THE COMPONENT(S) OF DEP RESPONSIBLE FOR IMMUNOTOXICITY

On the other hand, the components in DEP responsible for the enhancing effects on the model remain to be identified. DEP are complicated particles consisting of carbonaceous nuclei and a vast number of organic chemical compounds such as polyaromatic hydrocarbons, aliphatic hydrocarbons, heterocycles, and quinones. Previous studies have indicated that organic chemicals extracted from DEP result in induction of apoptosis [47], increase oxidative stress [48], and induce inflammatory cells [49-51] through the release of proinflammatory molecules [51,52] *in vitro*. Also, we have recently demonstrated that extracted organic chemicals from DEP rather than residual carbonaceous nuclei of DEP after extraction predominantly enhance antigen-related airway inflammation in mice [45]. However, detailed researches about the component(s) of DEP responsible for their effects on the respiratory system and/or pulmonary diseases remain unsatisfied especially *in vivo*.

Otherwise, a variety of quinones have been identified as DEP components [53,54]. Quinones themselves have toxicological properties to serve as alkylating agents and to interact with, for example, flavoproteins to generate Reactive Oxygen Species (**ROS**), which can lead to biological injury [55-58]. Phenanthraquinone (**PQ**) is one of quinones involved in DEP [54,55]. We have

recently shown that PQ itself induces lung inflammation [59] and aggravates antigen-related airway inflammation [60] in mice. Naphthoquinone (NQ; MW: 158) is another quinone involved in DEP [58,61]. Detailed chemical analysis has reported that approximately 13.7  $\mu\text{g}$  of NQ is contained in 1 g of DEP [58]. We have also shown that NQ deteriorates antigen-related airway inflammation in mice [62]. In brief, pulmonary exposure to NQ has aggravated antigen-related airway inflammation characterized by infiltration of eosinophils and lymphocytes around the airway and an increase in goblet cells in the bronchial epithelium in a dose-dependent manner with enhanced lung expressions of Th2 cytokines such as interleukin (IL)-4 and IL-5 [62]. We have recently shown that NQs differentially enhanced allergen sensitization in the context of the Th response such as Th1/2/17 against mononuclear cells, e.g., lymphocytes, and this may, at least in part, contribute to the immuno enhancement of DEP in allergic diseases [63]. Nonetheless, other chemicals composed in DEP should be elucidated for having potential in exacerbation of allergy in the future studies.

## CONCLUSION

Taking these *in vivo*, *in vitro*, and *ex vivo* studies from our laboratory as well as those from other ones into consideration, PM including DEP is likely to serve as stimulator/activator/enhancer of immune response related to allergy, in which various disrupting steps exist.

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