Exposure to allergen and diesel exhaust particles potentiates secondary allergen-specific memory responses, promoting asthma susceptibility

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Background: Exposure to traffic pollution particulate matter, predominantly diesel exhaust particles (DEPs), increases the risk of asthma and asthma exacerbation; however, the underlying mechanisms remain poorly understood.
Objective: We sought to examine the effect of DEP exposure on the generation and persistence of allergen-specific memory T cells in asthmatic patients and translate these findings by determining the effect of early DEP exposure on the prevalence of allergic asthma in children.
Methods: The effect of DEPs on house dust mite (HDM)–specific memory responses was determined by using an asthma model. Data from children enrolled in the Cincinnati Childhood Allergy and Air Pollution Study birth cohort were analyzed to determine the effect of DEP exposure on asthma outcomes.
Results: DEP coexposure with HDM resulted in persistent T_H2/T_H17 $CD127^+$ effector/memory cells in the lungs, spleen, and lymph nodes of adult and neonatal mice. After 7 weeks of rest, a single exposure to HDM resulted in airway hyperresponsiveness and increased T_H2 cytokine levels in mice that had been previously exposed to both HDM and DEPs versus those exposed to HDM alone. On the basis of these data, we examined whether DEP exposure was similarly associated with increased asthma prevalence in children in the presence or absence of allergen exposure/sensitization in the Cincinnati Childhood Allergy and Air Pollution Study birth cohort. Early-life exposure to high DEP levels was associated with significantly increased asthma prevalence among allergic children but not among nonallergic children.
Conclusion: These findings suggest that DEP exposure results in accumulation of allergen-specific T_H2/T_H17 cells in the lungs, potentiating secondary allergen recall responses and promoting the development of allergic asthma. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Allergic asthma, traffic pollution, house dust mite, diesel exhaust particle, memory, recall, children*

A recent comprehensive and systematic review of worldwide traffic emissions and health science by a special panel convened by the Health Effects Institute found sufficient evidence that exposure to traffic-related air pollution (TRAP) causes asthma exacerbation in children.[1] Diesel exhaust particles (DEPs) are a key component of traffic-related particulate matter and are the main contributor to TRAP-related asthma exacerbations in children.[2,3] These primary ultrafine DEPs (diameter, <1.0 μm) can reach the small airways, including the alveolar/gas exchange regions of the lung, exacerbating respiratory disease symptoms.[3] This exposure is highly significant because in large cities in North America, up to 45% of the population resides in zones that are most affected by TRAP.[1] Furthermore, more than 30% of schools are located in high TRAP exposure areas.[4] Even short-term exposure to high diesel traffic was able to reduce airway function in asthmatic patients.[5] Similarly, we recently reported that higher DEP exposure is associated with increased asthma severity in allergic children with asthma.[6]

Allergic asthma is generally regarded as a T_H2 disease characterized by increased eosinophil and T_H2 cytokine (IL-4, IL-5, and IL-13) levels, but severe asthma is often characterized by mixed T_H2/T_H17 responses.[7] In mice DEPs alone had no effect on airway hyperresponsiveness (AHR), but coexposure with house dust mite (HDM) exacerbated allergic airway responses, including allergen-specific IgE levels, eosinophilia, and AHR.[8] Transfer of antigen-specific IL-17A and IL-13 double-producing $CD4^+$ effector T cells into BALB/c mice triggered more severe inflammation on allergen challenge compared with transfer of conventional T_H2 or T_H17 cells, highlighting the potential role of this novel cell subset in allergic asthma severity.[9] Repeated DEP exposure promoted accumulation of T_H17 and T_H2/T_H17 coproducer cells in the lungs of exposed mice.[6] Neutralization of IL-17A alleviated DEP-mediated exacerbation of HDM-induced AHR, supporting a role for IL-17A in patients with severe asthma.[6]

Here, we examined the effect of DEP exposure on allergen-specific memory and recall responses. Generation and maintenance of memory T cells in the lungs are still poorly understood and have been studied predominantly in the context of

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Abbreviations used
AHR: Airway hyperresponsiveness
BALF: Bronchoalveolar lavage fluid
CCAAPS: Cincinnati Childhood Allergy and Air Pollution Study
CD62L: CD62 ligand
CDPM: Combustion-derived particulate matter
CLCA3: Chloride channel, calcium-activated, family member 3
DEP: Diesel exhaust particle
ECAT: Elemental carbon attributable to traffic (a proxy for DEP exposure)
HDM: House dust mite
IL-13R: IL-13 receptor
PE: Phycoerythrin
SPT: Skin prick test
TRAP: Traffic-related air pollution

viral infections.[10] Memory T cells are long-lived antigen-specific T cells that arise during expansion of effector T cells and survive the contraction phase of the effector response. Based primarily on studies focusing on $CD8^+$ memory T cells, 3 subpopulations have been described: central memory, effector memory, and tissue-resident memory T cells.[11] Memory T cells express high surface CD44 levels, and most express CD127, the receptor for IL-7, which plays a central role in $CD4^+$ T-cell homeostatic proliferation.[12] Central memory T cells circulate in secondary lymphoid organs (spleen and lymph nodes) and express CD62 ligand (CD62L) and CCR7. Effector/memory T cells downregulate CD62L and CCR7 to leave lymphoid tissues and then express tissue-specific integrins and chemokine receptors.[11,13] These infiltrating effector memory T cells differ from tissue-resident memory T cells, which are generated locally and express CD69, an early activation marker.[11,14,15]

In the present study we assess the effect of DEP-mediated asthma exacerbations on the generation and persistence of memory T cells. Because the nature and size of the effector response influences the nature and size of the memory T-cell pool,[16-18] we hypothesized that the increased accumulation of effector/memory T_H2 cells in the lungs of mice coexposed to HDM plus DEPs will result in the persistence of more HDM-specific memory T_H2 cells in the lungs on resolution of the effector T_H2 response, potentiating future recall responses. We further hypothesized that if DEP exposure potentiates recall responses to allergen, then early-life DEP exposure might promote the development of allergic asthma in children.

METHODS

For a complete description of the materials and methods used in the murine experiments, see the Methods section in this article's Online Repository at www.jacionline.org.

Cincinnati Childhood Allergy and Air Pollution Study cohort

The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) is an ongoing prospective birth cohort study that has been described previously.[19-22] For this analysis, 578 children from CCAAPS who had at least 1 skin prick test (SPT) response between the ages of 1 and 4 years and an asthma diagnosis at age 7 years were included. DEP exposure levels were estimated from the birth record address; a high DEP level was defined as the top quartile, as described previously.[21] Early-life aeroallergen sensitization and early HDM sensitization were defined as a positive SPT response for any of the 15 aeroallergens or a positive SPT response to HDM, respectively, at one examination between the ages of 1 and 4 years. Asthma diagnosis at age 7 years was based on reported symptoms and spirometric testing based on American Thoracic Society criteria.[23] This study was approved by the Institutional Review Boards of Cincinnati Children's Hospital Medical Center and the University of Cincinnati.

Statistical analysis

For the human data, statistical analyses were performed with SAS software (SAS Institute, Cary, NC). The differences in the proportion of children with asthma with respect to DEP exposure (low vs high) and early persistent atopy (no vs yes) were evaluated with χ^2 tests. A P value of less than .05 was considered significant. We also evaluated a logistic model adjusted for race, sex, and mother's education level. For the murine studies, statistical analyses were done with PRISM software (GraphPad Software, La Jolla, Calif). Statistical significance was assessed by using 1-way ANOVA, followed by a Bonferroni post test on the relevant groups.

RESULTS

DEP-associated neutrophilia persists after HDM-induced T_H2 responses return to baseline

First, we determined how coexposure to DEPs affects the resolution of HDM-induced lung inflammation by assessing eosinophil and T_H2 cytokine bronchoalveolar lavage fluid (BALF) levels 8 and 30 days after the last exposure (Fig 1, *A*). As we have previously shown,[6,24] DEP coexposure exacerbates HDM-induced lung inflammation (Fig 1, *B*). One week later, HDM-mediated induction of BALF eosinophilia had largely subsided, and DEP-related neutrophilia represented the major inflammatory cell type in the BALF (Fig 1, *C* and *D*). BALF levels of IL-5 and eotaxin-1 (CCL11) were also significantly ablated, whereas CXCL1 and CXCL5 levels remained increased 8 days after the last exposure (see Fig E1, *B* and *C*, in this article's Online Repository at www.jacionline.org). At 30 days after exposure, neutrophil BALF levels were still significantly increased in DEP-exposed mice (Fig 1, *C*). In contrast, eosinophils and T_H2 cytokines were no longer present in the BALF of HDM plus DEP–exposed mice (Fig 1, *D-F*). Exposure to DEPs alone did not induce AHR, but HDM and DEP coexposure significantly exacerbated AHR 24 hours after the last exposure (Fig 1, *G*). By 30 days after exposure, AHR had returned to baseline values (Fig 1, *G*). Consistent with decreasing BALF T_H2 cytokine levels and AHR, goblet cell numbers (as assessed based on chloride channel, calcium-activated, family member 3 [CLCA3; also known as gob-5] expression) and mucus production were also diminished (Fig 1, *H*, and see Fig E1, *D*). Taken together, these results suggest that allergic T_H2 response resolved within 1 month after the last exposure, even in mice coexposed to HDM and DEPs.

DEP and HDM coexposure promotes a persistent increase in lung effector/memory T_H2 cell numbers

Coexposure to HDM and DEPs significantly increased lung cell numbers compared with those in mice exposed to either DEPs or HDM alone (Fig 2, *A*). This accumulation of inflammatory cells in the lungs lasted for more than a week, but lung cell numbers returned to baseline within a month (Fig 2, *A*). Numbers of effector T cells, which were defined as $CD4^+CD44^+CD62L^-$ T cells, were significantly increased in mice coexposed to HDM and DEPs compared with those exposed to HDM alone (Fig 2,