

Correspondence

DEP-induced T_H17 response in asthmatic subjects

To the Editor:

We read with great interest the recent study by Brandt et al¹ about the key role of IL-17A and its producers such as T_H17 cells in diesel exhaust particle (DEP)-mediated severe allergic asthma. Although we appreciate their comprehensive work, we have few comments and queries for the article. (1) We feel as if there is something a little off with the involvement of IL-6 as a proinflammatory cytokine because its precise role in inflammation remains controversial. Qiu et al² have reported that IL-6 knockout ($-/-$) mice revealed enhanced airway inflammation after chronic exposure to aeroantigen as compared with wild-type (WT) littermates, implying its antiinflammatory potential. Also, we have previously shown that IL-6 is protective against pulmonary hemorrhagic injury induced by bacterial endotoxin, at least partly, via the modulation of proinflammatory cytokines and oxidative stress using IL-6 ($-/-$) mice on B6J129Sv strain.³ Obviously, in the experiments, we have of interest observed that IL-6 ($-/-$) mice reveal exaggerated lung inflammatory injury as compared with corresponding WT mice within 3 days after an intraperitoneal injection of endotoxin, whereas mortality at day 7 tends to be higher in WT than in IL-6 ($-/-$) mice (Inoue K, unpublished data, 2004), suggesting that the cytokine oppositely contributes to the fatal condition between relatively early phase and late one. We imagine that IL-6 has biphasic functions in inflammation during its pathological phases as Qiu et al² proposed, although as a matter of course the role might be different according to animal species and strains and model of inflammation (stimuli). (2) Signals provoking T_H17 response remain unresolved completely. There are many candidates concerning its differentiation/induction/amplification. The IL-17-IL-23 axis is also important for T_H17 -related pathophysiology, and IL-21 coupled with transforming growth factor beta promotes a transcriptional factor, RAR-related orphan receptor (ROR) γ t induction in T_H0 cells, which skew T_H17 differentiation

through the enhanced expression of the IL-23 receptor. We have previously shown that nanoparticle-rich DEP induce both IL-17A synthesis and ROR γ t activation in naive splenic mononuclear cells from atopy-prone animals,⁴ indicating that nanoparticle-rich DEP can activate the T_H17 reaction *in vitro*, at least in part, through the induction of ROR γ t. Therefore, we would like to know the status of these molecules mentioned above in their experimental studies. (3) As proposed in the study by Brandt et al, effects of particulate matters (PM) on allergic inflammation and airway hyperresponsiveness seem to be independent. We have previously elucidated effects of some particles intratracheally administered on cholinergic airway reactivity in asthmatic mice by means of flexiVent (see this article's [Methods](#) section in the Online Repository at www.jacionline.org). We selected and examined particulate component derived from DEP (washed DEP^{E1}), 2 different types (particle size, 14 nm and 56 nm) of carbon black nanoparticles (CBNPs^{E3}), 3 sizes (25, 50, and 100 nm) of polystyrene nanoparticles (PSNPs^{E5}), and 2 kinds (multiwalled and single-walled) of carbon nanotubes (CNTs^{E6,E7}). As a result ([Fig 1](#)), tissue elastance (H) was significantly worse and system resistance (R), compliance (C), and tissue damping (G) tended to be worse in the washed DEP and allergen group than in the allergen group. Both sizes of CBNPs enhanced cholinergic airway reactivity, as assessed by total respiratory system resistance (R), Newtonian resistance (Rn), C, elastance (E), G, and H except for G in 14 nm of CBNPs and allergen as compared with the values in allergen alone. All sizes of PSNPs examined did not influence these parameters as compared with those in the allergen group. Multiwalled CNTs tended to worsen E, C, Rn, and H without statistical significance, whereas single-walled CNTs tended to restore R, C, and G elicited by allergen exposure. However, we have previously confirmed that these CBNPs^{E4} and CNTs^{E6,E7} significantly aggravate allergic airway inflammation characterized by the infiltration of eosinophils and neutrophils in the bronchoalveolar lavage fluids and washed DEP^{E2} and PSNPs^{E5} did not on the same

	R	E	C	Rn	G	H	AI
washed DEP	↗	→	↗	→	↗	↑	→
CBNP (14 nm)	↗	↗	↗	↑	→	↗	↑
CBNP (56 nm)	↑	↗	↗	↑	↗	↗	↑
PSNP	→	→	→	→	→	→	↗
MWCNT	→	↗	↗	↗	→	↗	↑
SWCNT	↘	→	↘	→	↘	↗	↑

FIG 1. Effects of several types of particles on cholinergic airway reactivity in asthmatic mice. AI, Airway inflammation; C, compliance; CBNP (14 nm), carbon black nanoparticles with particle size of 14 nm; CBNP (56 nm), carbon black nanoparticles with particle size of 56 nm; E, elastance; G, tissue damping; H, tissue elastance; MWCNT, multiwalled carbon nanotubes; PSNP, polystyrene nanoparticles; R, total respiratory system resistance; Rn, Newtonian resistance; SWCNT, single-walled carbon nanotubes; washed DEP, particulate component derived from diesel exhaust particles. ↑, exacerbation/aggravation with statistical significance as compared with value of allergen alone. ↗, exacerbation/aggravation without statistical significance as compared with value of allergen alone. →, equivalent to value of allergen alone. ↘, amelioration/alleviation without statistical significance as compared with value of allergen alone.

allergic asthma protocol as the present one, implying that impacts of these PM are different among several pathogenic traits in asthma. (4) In their human study, can the concentration of DEP at $0.46 \mu\text{g}/\text{m}^3$ defined as “high DEP exposure” translate/convert to airborne PM concentration? Investigation concerning the correlation between high PM concentration ($15 \mu\text{g}/\text{m}^3$; yearly average limit set by the US Environmental Protection Agency) and the pathologic severity with serum IL-17A level might accentuate the role.

Ken-ichiro Inoue, MD, PhD^a
Michitaka Tanaka, PhD^a
Hirohisa Takano, MD, PhD^b

From ^athe Center for Medical Science, International University of Health and Welfare, Ohtawara, Japan; and ^bthe Graduate School of Engineering, Kyoto University, Kyoto, Japan. E-mail: kinoue@iuhw.ac.jp.

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METHODS

Animals

Male ICR mice aged 6 to 7 weeks (Japan Clea Co, Tokyo, Japan) were used in all experiments. They were fed a commercial diet (Japan Clea Co) and given water ad libitum. Mice were housed in an animal facility that was maintained at 24°C to 26°C with 55% to 75% humidity and a 12-hour light/dark cycle.

Particles

Preparation of DEP and washed DEP is previously described.^{E1,E2} Two sizes (14 and 56 nm) of CBNPs were purchased from degussa (Dusseldorf, Germany) and prepared for experiments as previously described.^{E3,E4} All sizes (25, 50, and 100 nm) of polystyrene nanomaterials were purchased as fluorescent latex particles (micromer-blue F plain) from Micromod (Rostock, Germany) and prepared as previously described.^{E5} Two types (multiwalled and single-walled) of CNTs were purchased from Bussan Nanotech Research (Ibaraki, Japan) and SES Research (Houston, Tex) and Carbon Nanotechnologies, Inc (Houston, Tex), respectively, and prepared as previously described.^{E6,E7}

Study protocol

In each experiment, mice were divided into the allergen (ovalbumin: Sigma Chemical Co, St Louis, Mo) group and the allergen and particle group and the study protocol complied with those in our previous studies concerning an allergic asthma model.^{E1-E7} All mice were examined for lung function 24 hours after the final intratracheal instillation. The study protocol followed the National Institutes of Health guidelines for the experimental use of animals. All animal studies were approved by the Institutional Review Board of the National Institute for Environmental Studies.

Analysis of lung function

Assessment of cholinergic airway/lung constrictor responsiveness was done with a computer-controlled small-animal ventilator (FlexiVent; Scireq, Montreal, Canada) as previously described.^{E1-E7} In brief, the mice were anesthetized with 0.1 mL per 10 g body weight of 40 mg/mL ketamine hydrochloride given intraperitoneally. Anesthesia was maintained by supplemental administration of 30% of the initial dose at approximately 25-minute intervals, as required. Mice were tracheostomized with a 5-mm section of metallic tubing and ventilated (FlexiVent) at 180 breaths/minute with a tidal volume of 10 mL/kg and a positive end-expiratory pressure of 3 cmH₂O.

Both the single-compartment model (using the snap shot method) and the constant-phase model (using the forced oscillation technique [FOT] method) of respiratory mechanics were applied to assess airway responses and lung function to methacholine. For the single-compartment model, total respiratory system resistance (R), elastance (E), and compliance (C) were determined essentially as described previously.^{E1,E3} For the constant-phase model, Newtonian resistance (R_n), tissue damping (G), and tissue elastance (H) were determined as described previously.^{E1,E3} All data points were determined by the

FlexiVent software (version 5.0) by using multiple linear regression to fit each data point to the single-compartment model or the constant-phase model, as appropriate. The lung volume history of the mice was standardized before the measurement of lung mechanics using 2 deep inflations. P and V data were generated by applying a 2-second sine wave volume perturbation (SW) with an amplitude of 0.2 mL and a frequency of 2.5 Hz. After 5 minutes of regular mechanical ventilation, the SW perturbation was applied 3 times and the average was taken to generate a baseline measurement. The respiratory system input impedance (Z_{rs}) was measured during periods of apnea using a 3-second signal containing 19 mutually prime sinusoidal frequencies ranging from 0.25 to 19.625 Hz; these maneuvers generated data that were fitted to the single-compartment model or the constant-phase compartment model, respectively. The averages of these measurements for each mouse served as its baseline values. Following the acquisition of baseline data, airway responsiveness to aerosolized methacholine (0.125–25 mg/mL saline; delivered by ultrasonic nebulizer) was assessed using both the snap shot method and the FOT method. Aerosols were delivered for 10 seconds with ventilation at 180 breaths/minute with a tidal volume of 4 mL/kg, after which the snap shot and the FOT methods were applied consecutively every 6 seconds for 5 minutes. Peak responses during each 5-minute period were determined, and only those values that had a coefficient of determination of 0.95 or more were used (n = 6–9 in each group).

Statistical analysis

Data were reported as mean ± SD. Differences were analyzed by using the Kruskal-Wallis test followed by the Mann-Whitney *U* test using the SPSS 8.0 statistical package for Windows (Chicago, Ill). Values of *P* < .05 were considered significant.

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