

Neurotrophin Mediation of Allergic Airways Responses to Inhaled Diesel Particles in Mice

Aimen K. Farraj,*†¹ Najwa Haykal-Coates,* Allen D. Ledbetter,* Paul A. Evansky,* and Stephen H. Gavett*

*Experimental Toxicology Division, U.S. EPA, Research Triangle Park, North Carolina 27711; and

†North Carolina State University, Raleigh, North Carolina 27695

Received June 14, 2006; accepted August 8, 2006

Neurotrophins, including nerve growth factor (NGF), partially mediate many features of allergic airways disease including airway hyperresponsiveness. Diesel exhaust particulates (DEP) associated with the combustion of diesel fuel exacerbate many of these allergic airways responses in humans. We tested the hypothesis that DEP-induced enhancement of allergic airways disease in a murine model is dependent on normal function of the low affinity pan-neurotrophin receptor $p75^{\text{NTR}}$, or tyrosine kinase A (trkA), the primary receptor for NGF. Ovalbumin (OVA)-sensitized and nonallergic BALB/c mice were intranasally instilled with anti- $p75^{\text{NTR}}$, anti-trkA, or vehicle, 1 h before OVA aerosol challenge, and then exposed nose-only to the particulate matter fraction that was less than 2.5 microns in aerodynamic diameter fraction of Standard Reference Material 2975 DEP (2.0 mg/m³) or filtered air for 5 h. One day later, DEP-exposed OVA-allergic mice had significantly greater increases in ventilatory responses to methacholine (Mch), but not increased lung resistance, suggesting that the airflow changes may have originated in the nasal passages. DEP-exposed OVA-allergic mice also had increased lung IL-4 levels relative to all other groups. The instillation of anti- $p75^{\text{NTR}}$ or anti-trkA completely reversed the DEP-induced increases in ventilatory responses and lung IL-4 protein to levels similar to control mice. OVA-allergic DEP-exposed mice treated with anti- $p75^{\text{NTR}}$ had significantly less lung resistance in response to Mch relative to OVA-allergic DEP-exposed mice treated with anti-trkA. The results of this study demonstrate that the enhancement of allergic airways responses by DEP exposure is partly dependent on neurotrophins in mice. In addition, neurotrophins that bind $p75^{\text{NTR}}$, but not trkA, may mediate pulmonary central airways and tissue resistance responses to allergen and DEP exposure.

This paper has been reviewed and approved for release by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency (EPA). Approval does not signify that the contents necessarily reflect the views and policies of the U.S. EPA, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

This research was funded in part by U.S. EPA North Carolina State University Cooperative agreement # CT829470.

¹ To whom correspondence should be addressed at Experimental Toxicology Division, U.S. EPA, 109 T.W. Alexander Drive, Mail Code: B143-01, Research Triangle Park, NC 27711. Fax: (919) 541-0026. E-mail: farraj.aimen@epa.gov.

Key Words: neurotrophins; $p75^{\text{NTR}}$; trkA; diesel particles; asthma exacerbation; airway physiology; Penh; airways resistance; lung mechanics; nose-only; BALB/c mice.

An important class of mediators known as neurotrophins, critical to nerve function, has recently drawn substantial interest in the field of airway biology. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3 (NT-3) are examples of neurotrophins, which are essential for neuronal growth and maintenance, neurotransmitter and neuropeptide production, and excitability (Olgart Hoglund *et al.*, 2002). Recently, neurotrophins and their receptors were found to be expressed in many cell types within the respiratory tract including the bronchial epithelium, pulmonary smooth muscle, mast cells, alveolar macrophages, eosinophils, T lymphocytes, ganglionic neurons, and fibroblasts (Ricci *et al.*, 2004). Many lines of evidence suggest that neurotrophins play a key role in asthma. In one study, asthmatics had elevated levels of NGF in their lung lining fluid (Virchow *et al.*, 1998). In addition, NGF, BDNF, and NT-3 were determined to be necessary for normal eosinophil function in patients with allergic bronchial asthma (Nassenstein *et al.*, 2003). The link between neurotrophins and asthma has also been established in animal models of allergen-induced asthma. For example, BDNF expression in the respiratory epithelium was upregulated in the lungs of allergic mice (Braun *et al.*, 1999). The overexpression of NGF in the lungs of mice caused an enhanced response to allergen challenge (Path *et al.*, 2002).

Exposure to diesel exhaust particulates (DEP) arising from the combustion of diesel fuel can exacerbate asthma. Large vehicles such as buses and trucks and many industrial vehicles are major sources of this type of air pollution. DEP consists of a carbonaceous core with a large surface area that has heavy metals such as iron and polyaromatic hydrocarbons such as quinones adsorbed to it. DEP is a major source of ambient particulate matter fraction that was less than 2.5 microns in aerodynamic diameter (PM_{2.5}) (Pandya *et al.*, 2002; Sydbom *et al.*, 2001). Recent epidemiologic and controlled human exposure studies have demonstrated a link between vehicular

traffic pollution and deleterious respiratory effects including asthma (Nightingale *et al.*, 2000; Nordenhall *et al.*, 2001; Ostro *et al.*, 1999; Svartengren *et al.*, 2000). Several animal studies have also correlated diesel particulate exposure to asthma exacerbation (Dong *et al.*, 2005; Siegel *et al.*, 2004; Steerenberg *et al.*, 2003). Multiple mechanisms of DEP-induced exacerbation of asthma have been suggested including enhanced production of reactive oxygen species, direct immune effects such as enhanced IgE levels and stimulation of eosinophils, and adjuvant effects in the context of allergen exposure (Becker *et al.*, 2002; Ormstad, 2000; Reidl and Diaz-Sanchez *et al.*, 2005). A recent finding suggests that neurotrophins may also be involved in DEP-induced exacerbation of asthma. In one study, exposure to road traffic enhanced allergen-induced increases in plasma levels of NGF in subjects with atopic dermatitis (Kimata, 2004). In addition, the cell types within the airways that are affected by DEP, including eosinophils, macrophages, lymphocytes, and the airway epithelium, produce neurotrophins and/or express neurotrophin receptors. Also, DEP exposure enhances features of asthma that have been linked to NGF function, such as airway hyperresponsiveness (Svartengren *et al.*, 2000). The importance of neurotrophins in DEP-induced exacerbation of asthma, however, is unknown.

The primary receptor for NGF in the airways is tyrosine kinase A (trkA) (Ricci *et al.*, 2004). The pan-neurotrophin receptor, p75^{NTR}, is a low affinity receptor for all neurotrophins (Levitan and Kaczmarek, 1997). The p75^{NTR} receptor potentiates signaling through the trk receptors trkA, trkB, and trkC, which are the primary receptors for NGF, BDNF, and NT-3, respectively, and is thus required for normal activation of neurotrophin signaling (Levitan and Kaczmarek, 1997). Recently, we demonstrated that blockade of the p75 receptor inhibits the DEP-induced exacerbation of allergic airway responses in C57/BL6 mice (Farraj *et al.*, 2006). In the present study, we compared the effects of blockade of the p75 receptor to that of the trkA receptor in a model of DEP-induced enhancement of allergic airways disease using BALB/c mice. In addition, we assessed pulmonary function using two different methodologies: whole-body plethysmography (WBP) and lung mechanics. Mice were sensitized and challenged with ovalbumin (OVA) in a manner that elicited a mild allergic response rather than a strong response to prevent the potential masking of any DEP-induced effect. The mice were then exposed to DEP to enhance the characteristic features of allergic airways disease. DEP-exposed mice were intranasally instilled with an antibody against the p75^{NTR} receptor or the trkA receptor. Airway physiology, inflammation, and cytokine responses were assessed to determine the importance of normal p75^{NTR} or trkA function in this model of DEP-induced exacerbation of allergic airways disease.

METHODS

Animals. Young adult (6-week-old) male BALB/c mice were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were housed in plastic cages

on beta-chip bedding in groups of three per cage, maintained on a 12-h light/dark cycle at approximately 22°C and 50% relative humidity in our Association for Assessment and Accreditation of Laboratory Animal Care–approved facility, and held for a minimum of 4 days before treatment. Food (Prolab RMH 3000; PMI Nutrition International, St Louis, MO) and water were provided *ad libitum*. Mice were randomized by weight using a validated weight randomization program to assign mice to exposure groups.

Experimental design. Figure 1 depicts the exposure regimen used for mouse exposure. On day 0, mice were administered an ip injection of 20 µg of OVA in an aluminum hydroxide adjuvant gel vehicle (Sigma Chemical, St Louis, MO) or vehicle alone. Two weeks later, the mice were intranasally instilled with 50 µl of rabbit anti-mouse p75^{NTR} neurotrophin receptor polyclonal antibody (Chemicon, Temecula, CA), anti-mouse trkA NGF receptor antibody (Santa Cruz, Santa Cruz, CA), or sterile saline vehicle alone. Antibodies were diluted 1:50 following a method described by Kerzel *et al.* (2003). One hour later, all mice were challenged with a 1% OVA aerosol for 1 h. The mean concentration for OVA ranged from 208 to 214 mg/m³ for all of the OVA challenges. The mass median aerodynamic diameter (MMAD) ranged from 0.74 to 1.57 µm and the geometric standard deviation (GSD) was 4.47. One hour after OVA challenge, the mice were exposed for 5 h nose-only to the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2975 DEP. Studies were conducted in two separate cohorts of mice. In the first cohort, 1 day after DEP exposure, respiratory responses were measured using WBP in all 12 group combinations (*n* = 8/group). In the second cohort, 1 day after the same treatment protocol, lung mechanics were assessed in five select groups: (1) alhydrogel sensitization/saline instillation/air exposure; (2) OVA/saline/air; (3) OVA/saline/DEP; (4) OVA/anti-p75^{NTR}/DEP; and (5) OVA/anti-trkA/DEP (*n* = 7 or 8 per group).

Exposure to diesel exhaust particles. SRM 2975, collected by a filtering system of a diesel-powered industrial forklift, was obtained from the National Institute of Standards and Technology (Gaithersburg, MD). A DEP concentration of 2.0 mg/m³ was selected as the exposure concentration because it optimally enhanced the allergic airways response, particularly ventilatory responses. The mean concentration for DEP ranged from 1.78 to 2.18 mg/m³. The MMAD was 1.47 µm and the GSD was 2.75. Mice were exposed to DEP or filtered air in two separate 52-port nose-only flow-by inhalation chambers (Lab Products, Seaford, DE) using a previously described exposure system (Ledbetter *et al.*, 1998). Particles are carried through a charge neutralizer and 2.5-µm cut-point cyclone to remove particles larger than PM_{2.5} before entering the inlet of the nose-only chamber. Mice were acclimated to the nose-only exposure tubes prior to exposure. Dust concentration was determined gravimetrically on Teflon filters once per hour during the 5-h exposure period (45 mm diameter with 1 µm pore size; VWR Scientific, West Chester, PA), and real-time PM concentration was estimated with an aerosol monitor (Dust Track;

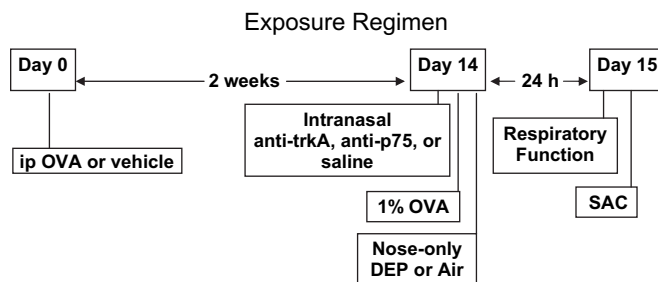


FIG. 1. Timeline of the exposure regimen for BALB/c mice. Mice were sensitized with an ip injection of OVA or administered alhydrogel vehicle alone. Two weeks later, the mice were intranasally instilled with 50 µl of anti-p75 or anti-trkA and then challenged with a 1% OVA aerosol 1 h later. One hour after challenge, the mice were exposed nose-only to NIST SRM-2975 DEP at a concentration of 2.0 mg/m³. One day later, respiratory effects were measured by WBP or lung mechanics followed by sacrifice (SAC).

TSI, Inc., St Paul, MN) on the chamber exhaust. The particle size was determined gravimetrically using a Mercer cascade impactor (Intox Products, Albuquerque, NM).

Whole body plethysmography. We measured respiratory responsiveness to increasing concentrations of aerosolized methacholine (Mch) in unanesthetized, unrestrained mice in a 12-chamber WBP system (Buxco Electronics, Sharon, CT) 24 h after DEP exposure. We assessed the airway response 24 h after OVA challenge because we determined that both respiratory responsiveness and airway inflammation were elevated at this time in pilot studies. Enhanced pause (Penh) was used as an index of ventilatory timing as previously described (Gavett *et al.*, 2003; Hamelmann *et al.*, 1997). After measuring baseline parameters for 7 min, an aerosol of saline or Mch in increasing concentrations (16, 32, and 64 mg/ml) was nebulized through an inlet of the chamber. The response to saline or Mch was measured over the aerosolization period (1 min), an aerosol drying step (2 min), and an additional 4-min period.

Lung mechanics. Mice were anesthetized with urethane (1.5 g/kg, ip), tracheotomized with an 18-gauge cannula, placed over a heating pad, and ventilated with a constant flow of air (flexiVent; Scireq, Montreal, Canada) at a frequency of 150/min (40% inspiratory cycle), tidal volume of 7.5 ml/kg, and positive end-expiratory pressure of 2 cm H₂O. Mice were given pancuronium bromide (0.8 mg/kg, ip, Sigma-Aldrich, St Louis, MO) to block spontaneous breathing. Increasing doses of Mch (1 mg/ml; 31.6, 100, 316, 1000, 3162 mg/kg) were infused into the jugular vein over 2 s with a syringe pump, allowing an interval of at least 2 min for recovery. Measures of pressure, volume, and flow were used to obtain lung resistance (R_L) and elastance (E_L) using the linear single compartment model: $\Delta P_L = E_L \cdot V_L + R_L \cdot dV_L/dt$, where P_L = lung pressure and V_L = lung volume. We also measured central airways (Newtonian) resistance (R_n), tissue damping (G_T), and tissue elastance (H_T) by the forced oscillatory technique, fitting the data to the constant phase model (Hantos *et al.*, 1992): $Z = R_n + i2\pi fI + (G_T - iH_T)/(2\pi f)^\alpha$, where Z = respiratory input impedance, f = breathing frequency, I = airways gas inertance, and $\alpha = (2/\pi) \cdot \arctan(H_T/G_T)$. G_T is associated with tissue resistance and reflects energy dissipation in the lung peripheral tissues, whereas H_T reflects energy storage in the lung tissues (Gomes *et al.*, 2000). Six measures each of single compartment model and constant phase model parameters were measured within 90 s following each dose of Mch. After subtracting baseline values taken prior to each dose, average parameter values following each Mch dose were analyzed for statistical differences.

Bronchoalveolar lavage. The mice that underwent WBP were deeply anesthetized with urethane (1.5 g/kg ip) 1 h later. Blood samples were taken from the abdominal aorta and serum samples were collected and stored at -80°C . The abdominal aorta and renal artery were then severed to exsanguinate the mice. After exsanguination, the trachea was cannulated and the lungs were lavaged with two aliquots of Ca^{2+} , Mg^{2+} , and phenol red-free Hank's balanced salt solution (HBSS, 35 ml/kg; Life Technologies, Bethesda, MD). Approximately 85% of the total instilled volume was recovered in all treatment groups. The bronchoalveolar lavage (BAL) fluid was maintained on ice and centrifuged at $360 \times g$ for 10 min at 4°C . BAL cells were resuspended in 1 ml of HBSS and counted (Z1; Coulter, Hialeah, FL). Cytospin preparations of BAL cell samples were made and stained with Wright-Giemsa using an automated slide stainer (Hematek 2000; Miles, Inc., Elkhart, IN). Cell differentials and percentages were determined by counting 500 cells per slide. Assays for the Thelper 2 (Th2) cytokines interleukin (IL)-4, IL-5, and IL-13 were carried out on an aliquot of BAL supernatant using commercially available ELISA (enzyme-linked immunosorbent assay) kits (R&D Systems, Minneapolis, MN). The minimum amount detectable for each cytokine ELISA was 7.8 pg/ml for IL-4 and IL-13 and 15.6 pg/ml for IL-5. Samples with values below the minimum detectable level were assigned that value for statistical analysis.

Histopathology. Following BAL and removal of the right lung lobes (snap-frozen for future Western blotting to assess presence of neurotrophins and their receptors), the left lung lobe was intratracheally perfused with 4% paraformaldehyde at a constant intra-airway pressure of 30 cm of fixative. After 1 h,

the trachea was ligated, and the inflated left lung lobe was immersed in a large volume of the same fixative for 4 h. The lungs were then placed in 70% ethanol at 4°C . After fixation, the left lung lobe was microdissected along the axial airways, and sections were then excised at the level of the fifth airway generation as described previously (Steiger *et al.*, 1995). Fixed lungs were processed to paraffin blocks, sectioned at a thickness of 5 μm , placed on glass slides, and stained with hematoxylin and eosin or Alcian blue/periodic acid Schiff's sequence for intraepithelial mucosubstances.

Serum IgE. OVA-specific serum IgE was measured using a 96-well Nunc ELISA plate (Nalge Nunc International, Rochester, NY) coated with 0.5 mg/ml OVA (Sigma-Aldrich) and incubated overnight at 4°C . After washing, the plates were incubated in 3% bovine serum albumin at 37°C for 1 h (Calbiochem, La Jolla, CA). Serum samples at 1:10 dilution or anti-OVA mouse IgE standards (Serotec, Oxford, UK) were then added followed by incubation at 37°C for 1 h. After washing, biotinylated anti-mouse IgE (Pharmingen, San Diego, CA) was then added (2 $\mu\text{g}/\text{ml}$) and allowed to incubate at 25°C for 1 h. After washing, 1.5 $\mu\text{g}/\text{ml}$ of streptavidin peroxidase (Pharmingen) was added followed by incubation at 25°C for 1 h. After washing, tetramethylbenzidine substrate (Alpha Diagnostic International, San Antonio, TX) was added to produce a color reaction. After color development, the reaction was terminated by the addition of H_2SO_4 (Alpha Diagnostic International). Optical density was determined at 450 nm using a SpectraMax 340pc plate reader (Molecular Devices, Sunnyvale, CA). The mean concentrations were determined using SoftmaxPro software (Molecular Devices). The assay limit of detection was 15.6 ng/ml.

Statistical analysis. All statistical analyses were performed using SAS procedures (Cary, NC). Physiological responses recorded repeatedly for each animal as average Penh and lung function parameters were analyzed by a repeated-measures mixed models methodology (PROC MIXED; SAS). The mixed model methodology enables the user to directly address the covariance structure and provides valid standard errors and efficient statistical tests. All vehicle- and OVA-sensitized groups were analyzed together and compared to each other. Individual measurements measured once for each animal (cell differentials, serum IgE, cytokines) were analyzed as a univariate variable with one-way analysis of variance. Tukey's multiple range comparison test was used for group comparisons. Group differences were considered significant if the test statistic type II error was less than 0.05.

RESULTS

Whole-Body Plethysmography

Increases in expiratory time and peak expiratory flow and decreases in inspiratory time and peak inspiratory flow contribute to increases in Penh. These changes in ventilatory timing may coincide with airflow obstruction or increased airway resistance at the level of the nasal, tracheobronchial, or pulmonary regions (Hamelmann *et al.*, 1997). One day after allergen challenge and exposure to DEP, there were no significant differences in average baseline Penh values among any of the treatment groups (Fig. 2). In vehicle-sensitized mice, exposure to DEP or treatment with antibodies against p75^{NTR} or trkA had no significant effect on Mch aerosol-induced increases in Penh (Fig. 2A). OVA-sensitized air-exposed mice were not significantly more responsive to Mch aerosol than vehicle-sensitized air-exposed mice (Fig. 2B). In contrast, exposure to DEP significantly enhanced Mch aerosol-induced Penh responses in OVA-sensitized mice in comparison with

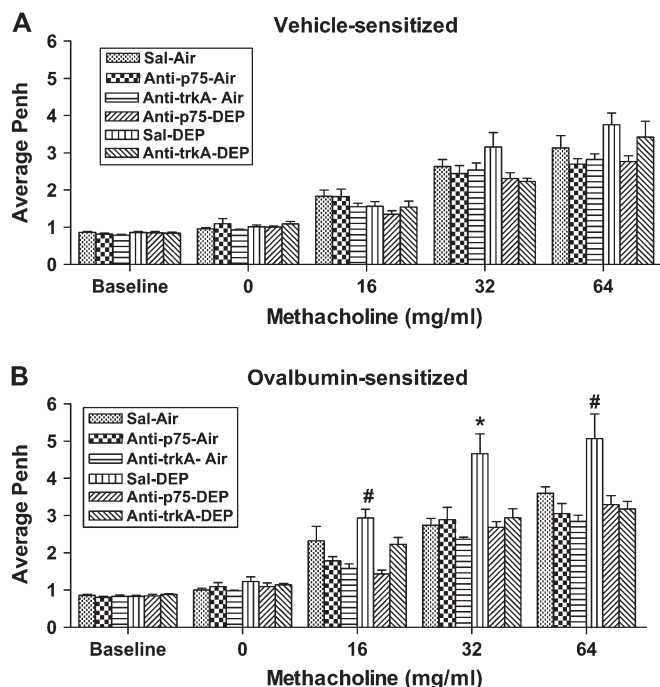


FIG. 2. Average Penh values were measured 24 h after DEP exposure in alhydrogel vehicle-sensitized (A) or OVA-sensitized mice (B) exposed to increasing concentrations of Mch. Bars represent average Penh values $\pm$ standard error of the mean ($n = 8$ per group). Sal = saline. DEP = NIST SRM-2975 diesel exhaust particles. #Significantly greater than all groups within same concentration of Mch, except Sal-Air ($p < 0.05$). *Significantly greater than all other groups at 32 mg/ml Mch ($p < 0.05$).

all other groups (Fig. 2B). At 32 mg/ml Mch, DEP-exposed OVA-sensitized mice had a 70% greater average Penh than air-exposed OVA-sensitized mice ($p < 0.05$). Administration of anti-p75^{NTR} or anti-trkA to OVA-sensitized mice completely reversed the DEP-induced increases in Penh ($p < 0.05$). Responses to Mch aerosol in all antibody-treated OVA-sensitized groups were similar to responses in air-exposed OVA-allergic mice instilled with saline vehicle only (Fig. 2B).

Lung Function in Ventilated Mice

Recent studies show that Penh is not always predictive of airway hyperreactivity (Bates *et al.*, 2004). Therefore, we compared our findings using WBP with measurements of lung mechanics parameters in ventilated mice following treatment with neurotrophin antibodies and exposure to OVA and DEP. The constant phase model of the lung was used to distinguish between mechanical properties of the central airways (R_n , I) and peripheral lung tissues (G_T , H_T) (Hantos *et al.*, 1992). Central airways resistance (R_n) was increased 62% in OVA-sensitized air-exposed mice compared with vehicle-sensitized air-exposed mice following the highest dose of Mch but this increase was not statistically significant (Fig. 3A). In contrast to WBP Penh, DEP exposure in OVA-allergic mice did not further enhance R_n responses. Treatment with anti-p75^{NTR}

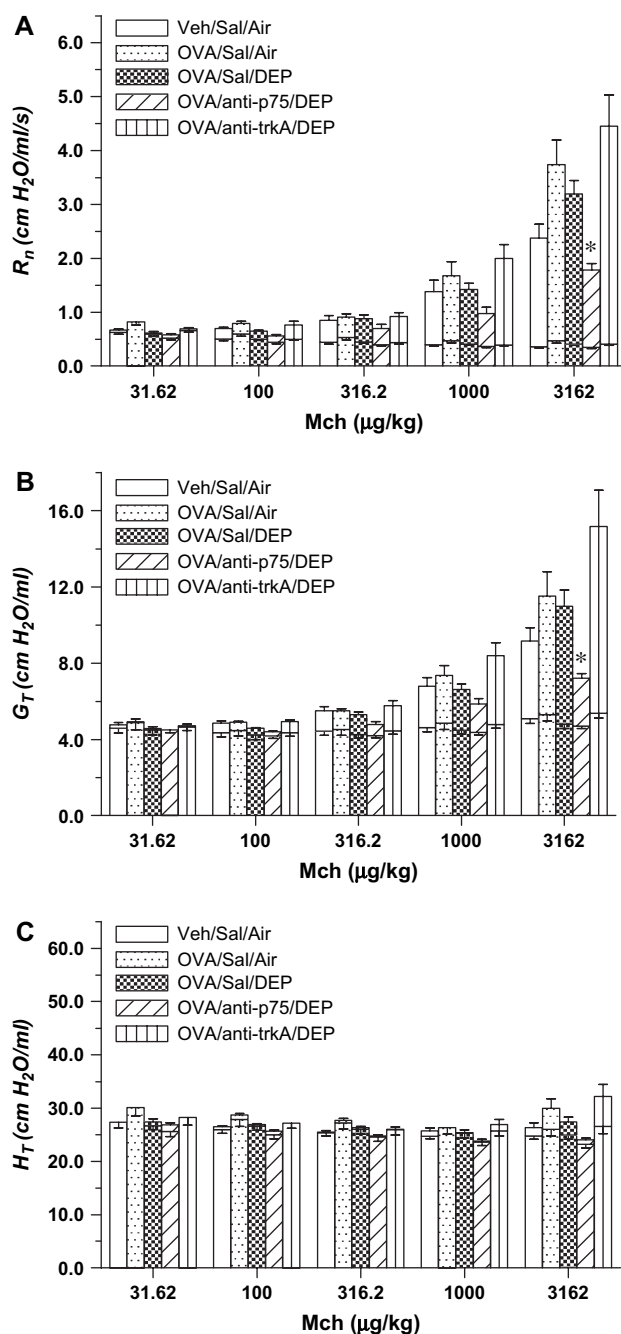


FIG. 3. Central airways (R_n ; panel A) and lung tissue (G_T , H_T ; panels B, C) mechanical properties were evaluated in mice treated with increasing doses of iv Mch. Data show means and standard error of the mean of baseline values (indicated by lines within bars) and Mch-induced responses stacked over baseline values ($n = 7-8$ per group). Sal = saline; Veh = alhydrogel vehicle. *Significantly less than OVA/anti-trkA/DEP group ($p < 0.05$).

decreased R_n responses in OVA-sensitized DEP-exposed mice to levels comparable to vehicle-sensitized air-exposed mice. Treatment with anti-trkA did not significantly alter R_n responses in OVA-sensitized DEP-exposed mice. The R_n response in OVA-sensitized DEP-exposed mice treated with

anti-p75^{NTR} was, however, significantly less than the R_n response in OVA-sensitized DEP-exposed mice treated with anti-trkA ($p < 0.05$). Similar response patterns were observed with tissue resistance (G_T ; Fig. 3B), and with the single compartment model parameters of lung resistance and elastance (R_L , E_L ; data not shown). The constant phase model parameter of tissue elastance (H_T ; Fig. 3C) was not significantly affected by any treatments or by increasing Mch dose, indicating the development of significant regional ventilation inhomogeneity during bronchoconstriction (Hantos *et al.*, 1992). Ventilation inhomogeneity may arise from unequal constriction of lung units caused by increases in tissue resistance (G_T) without corresponding increases in tissue stiffness (H_T) (Lutchen *et al.*, 1996).

Airway Pathology

OVA-sensitized, air-exposed mice had small increases in intraepithelial mucus in the epithelium lining the airway lumen of the main axial airway of the left lung lobe relative to vehicle-sensitized, air-exposed mice (Fig. 4). This epithelial change was accompanied by a mild influx of inflammatory cells that consisted of eosinophils and mononuclear cells including lymphocytes. DEP exposure did not enhance the severity of the OVA-induced airway pathology. DEP-laden macrophages were found in the cytopins of the lavage fluid samples, but were not apparent in the histopathologic examination of the lung tissue suggesting that they may have been washed out by the lavage procedure. Anti-p75^{NTR} or anti-trkA administration did not influence airway morphology.

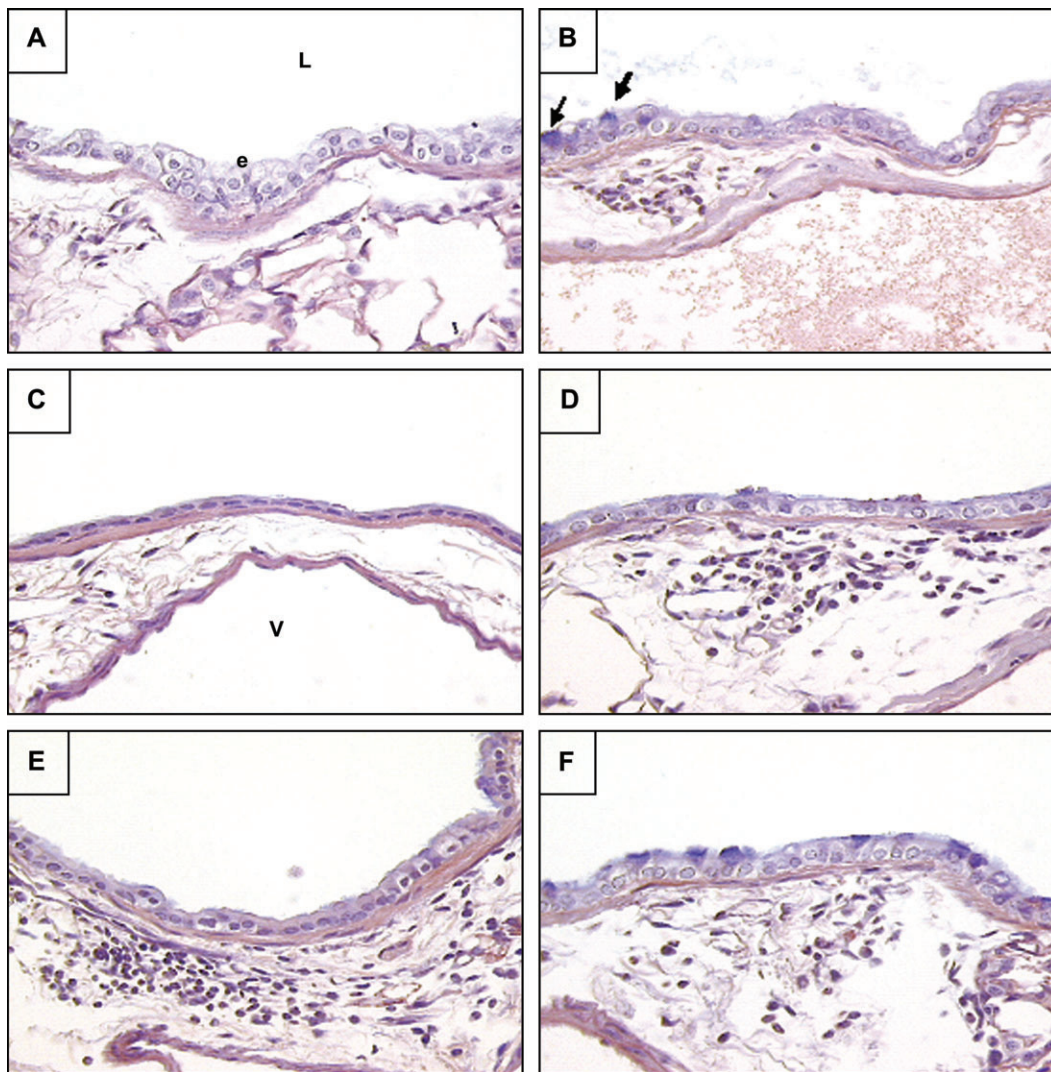


FIG. 4. Light photomicrographs (captured at $\times 40$ magnification) of the conducting airways of the mouse lung from the following groups: alhydrogel sensitized/saline instilled/air exposed (A); OVA sensitized/saline instilled/air exposed (B); alhydrogel sensitized/saline instilled/DEP exposed (C); OVA sensitized/saline instilled/DEP exposed (D); OVA sensitized/anti-p75^{NTR} instilled/DEP exposed (E); and OVA sensitized/anti-trkA instilled/DEP exposed (F). Tissues were stained with Alcian Blue/Periodic Acid Schiff's Sequence. L = airway lumen, e = airway epithelium, arrow = mucosubstances, v = blood vessel.

Cells in Lavage Fluid

DEP exposure in vehicle-sensitized mice significantly enhanced the number of macrophages in the lavage fluid by 92% relative to air-exposed vehicle-sensitized mice (Fig. 5A; $p < 0.05$). Anti-trkA or anti-p75^{NTR} administration significantly suppressed the DEP-induced increase in macrophages in vehicle-sensitized mice to levels similar to those of air-exposed vehicle-sensitized mice ($p < 0.05$). OVA-sensitized air-exposed mice had a 142% increase in the number of macrophages in the BAL fluid relative to air-exposed vehicle-sensitized mice (Fig. 5B vs. Fig. 5A; $p < 0.05$). Anti-trkA or anti-p75^{NTR} administration reversed the increase in macrophages in OVA-sensitized air-exposed mice to control levels ($p < 0.05$). DEP coexposure significantly decreased the number of macrophages in OVA-sensitized mice to control levels ($p < 0.05$). There was no effect of anti-trkA or anti-p75^{NTR} treatment on macrophage numbers in OVA-sensitized DEP-exposed mice (Fig. 5B).

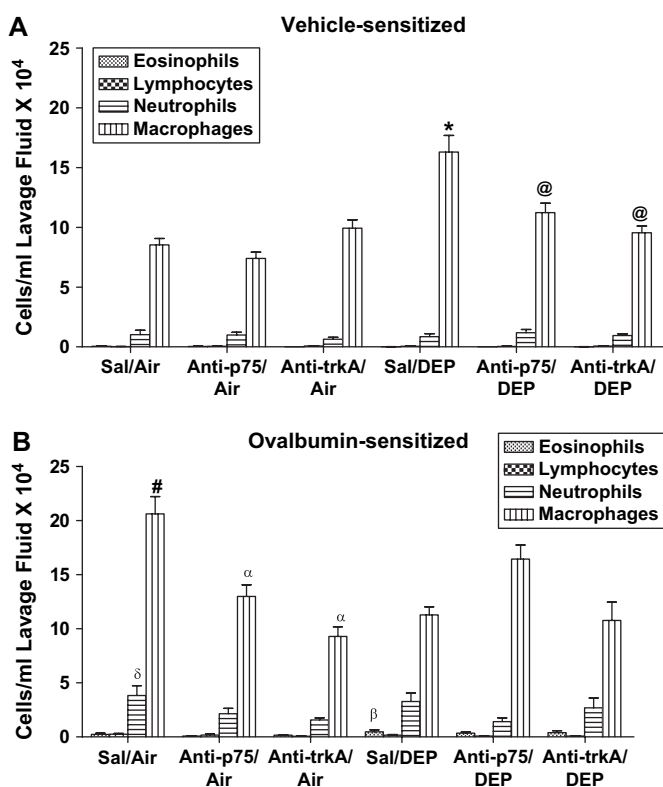


FIG. 5. Cells in BAL fluid from vehicle-sensitized (A) or OVA-sensitized (B) mice. Lavage fluid was collected 24 h after DEP exposure. Bars represent average cell number per ml lavage fluid $\pm$ standard error of the mean ($n = 8$ per group). *Significantly greater than all other vehicle-sensitized groups in the number of macrophages ($p < 0.05$). @Significantly less than the vehicle-sensitized Sal-DEP group in the number of macrophages ($p < 0.05$); Sal = saline. #Significantly greater than all other OVA-sensitized groups in the number of macrophages ($p < 0.05$). ^αSignificantly less than the OVA-sensitized Sal-Air group in the number of macrophages ($p < 0.05$). ^βSignificantly greater than all vehicle-sensitized groups in the number of eosinophils ($p < 0.05$). ^δSignificantly greater than all vehicle-sensitized groups in the number of neutrophils ($p < 0.05$).

OVA-sensitized air-exposed mice had a 90% increase in the number of eosinophils in the BAL fluid relative to air-exposed vehicle-sensitized mice but this was not statistically significant (Fig. 5B vs. Fig. 5A; $p < 0.05$). DEP coexposure caused a 110% increase in the number of eosinophils in OVA-sensitized mice relative to OVA-sensitized air-exposed mice but this difference was also not statistically significant. The number of eosinophils in the OVA-sensitized DEP-exposed mice was significantly greater than the number of eosinophils in the vehicle-sensitized air-exposed mice (Fig. 5B vs. Fig. 5A; $p < 0.05$). There were no significant effects of anti-trkA or anti-p75^{NTR} treatment on eosinophil numbers in OVA-sensitized mice. OVA-sensitized air-exposed mice had a 347% increase in the number of neutrophils in the BAL fluid relative to air-exposed vehicle-sensitized mice (Fig. 5B vs. Fig. 5A; $p < 0.05$). DEP exposure did not significantly affect neutrophil numbers in the lavage fluid of vehicle- or OVA-sensitized mice. There were no significant effects of anti-trkA or anti-p75^{NTR} treatment on neutrophil numbers in OVA-sensitized mice. There were no significant effects of OVA and/or DEP exposure on the number of lymphocytes in the lavage fluid.

Cytokines

OVA sensitization in air-exposed mice did not result in a significant increase in IL-4 protein levels in the BAL fluid relative to the vehicle-sensitized, air-exposed mice (Fig. 6). DEP exposure in OVA-sensitized mice caused a five-fold increase in the levels of IL-4 protein in the BAL fluid relative to the vehicle-sensitized, air-exposed mice ($p < 0.05$). Administration of anti-trkA or anti-p75^{NTR} significantly inhibited the DEP-induced increase in IL-4 protein in the BAL fluid in OVA-sensitized mice to levels similar to those of the air-exposed controls ($p < 0.05$). OVA sensitization in air-exposed mice did not result in a significant change in the levels of IL-5 or IL-13 protein relative to vehicle-sensitized, air-exposed mice. Anti-p75^{NTR} or anti-trkA administration and/or DEP exposure also had no significant effect on IL-5 or IL-13 levels.

Serum IgE

OVA sensitization caused a 10-fold increase in OVA-specific serum IgE levels relative to the vehicle controls in air-exposed mice (Fig. 7; $p < 0.05$). There were no significant effects of DEP, anti-p75^{NTR}, or anti-trkA treatment on serum IgE levels.

DISCUSSION

A hallmark feature of asthma is airway hyperresponsiveness. Experimental data show that DEP exposure enhances airway hyperresponsiveness in asthmatics. For example, the increased airway resistance and decreased lung function in asthmatics exposed to exhaust in a Stockholm tunnel were associated with high PM_{2.5} levels (Svartengren *et al.*, 2000). In addition, diesel

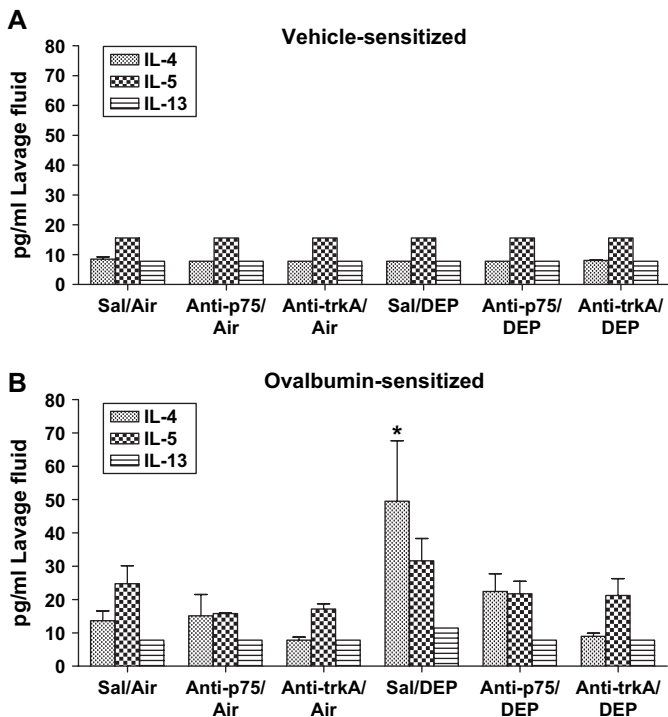


FIG. 6. IL-4, IL-5, or IL-13 protein levels in BAL fluid from vehicle-sensitized (A) or OVA-sensitized (B) mice. Lavage fluid was collected 24 h after DEP exposure. Bars represent average amounts of cytokine in pg per ml lavage fluid $\pm$ standard error of the mean ($n = 8$ per group). *Significantly greater than all vehicle- and OVA-sensitized groups in the levels of IL-4 ($p < 0.05$). Sal = saline.

particulate exposure in mice enhanced allergen-induced airway responsiveness (Takano *et al.*, 1998b). Neurotrophins have been shown to mediate airway hyperresponsiveness. For example, blockade of NGF prevented the increase in pulmonary airway resistance after allergen exposure in mice (Glaab *et al.*, 2003). The role of neurotrophins in airway responses to al-

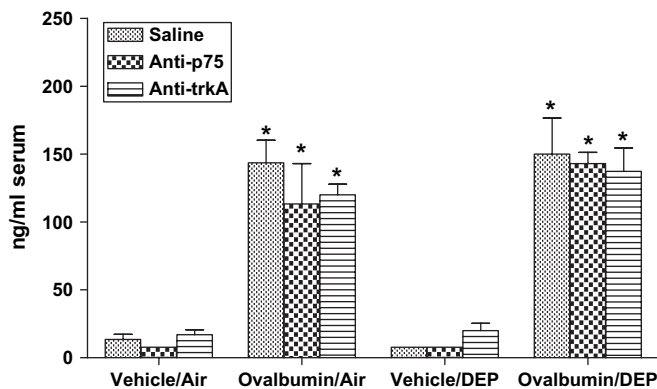


FIG. 7. OVA-specific serum IgE levels 24 h after DEP exposure in anti-p75 or anti-trkA-treated mice. Bars represent average amounts of IgE (ng/ml serum) $\pm$ standard error of the mean ($n = 8$ per group). *Significantly greater than all vehicle-sensitized groups ($p < 0.05$).

lergens and air pollutants, however, is poorly understood. This report demonstrates that neurotrophins are involved in the enhancement of allergic airways disease by inhaled DEP in a murine model of asthma.

Changes in ventilatory timing, including increases in expiratory time and peak expiratory flow and decreases in inspiratory time and peak inspiratory flow, contribute to increases in the empirically derived parameter Penh (Hamelmann *et al.*, 1997). Recently, it has been shown that changes in Penh could result from changes in breathing patterns distinct from changes in airflow obstruction (Bates *et al.*, 2004). However, strong correlations between increased Penh and increased airway resistance were found in direct comparisons of the techniques (Finotto *et al.*, 2001; Hamelmann *et al.*, 1997; Krane *et al.*, 2001). WBP and the assessment of lung mechanics provide different types of information. Penh was used as an indicator of both nasal and pulmonary airway changes. Airway resistance measurements from a cannulated trachea assess airflow obstruction due to central airway and peripheral tissue responses in the lung. In the present study, DEP exposure enhanced average Penh values in OVA-sensitized mice. In contrast, DEP did not significantly enhance airways resistance responses to Mch in OVA-sensitized ventilated mice. It is possible that DEP exposure may have caused airflow obstruction in the nasal airway of OVA-sensitized mice, which is bypassed in the assessment of lung mechanics, thus accounting for the enhancement in ventilatory responses. Further studies, however, are required to examine the contribution of nasal airflow changes to the ventilatory changes caused by DEP exposure. The intranasal instillation of anti-trkA or anti-p75^{NTR} attenuated the DEP-induced increase in Penh to control levels, suggesting that NGF and potentially other neurotrophins may be involved in the DEP-induced enhancement of nasal airflow obstruction. This is similar to findings in our previous study where anti-p75 instillation inhibited the DEP-induced increase in Penh in OVA-sensitized C57/Bl6 mice (Farraj *et al.*, 2006). OVA-sensitized mice treated with anti-p75^{NTR}, however, had significantly less lung resistance than OVA-sensitized mice treated with anti-trkA. These results suggest that neurotrophins other than NGF, such as BDNF or NT-3, that also bind the pan-neurotrophin receptor p75^{NTR}, may be involved in this mild lung response to OVA.

T-helper 2 lymphocytes secrete a battery of cytokines including IL-4, IL-5, and IL-13 that are responsible for mediating many of the pathophysiologic features characteristic of allergen-induced asthma (Frew, 1996; Yssel and Groux, 2000). DEP exposure enhanced Th2 cytokine production in the airways of humans exposed to ragweed allergen (Diaz-Sanchez *et al.*, 1997). Th2 cytokine expression is likely influenced by neurotrophins. T lymphocytes express the trkA receptor and produce both NGF and BDNF (Braun *et al.*, 1999; Ricci *et al.*, 2004). In the present study, DEP exposure enhanced the levels of IL-4 in the lavage fluid. The intranasal instillation of anti-p75^{NTR} or anti-trkA attenuated the DEP-induced

increase in IL-4. This finding suggests that the mechanism of DEP-induced increases in IL-4 may be dependent on normal neurotrophin function. It is worthwhile to note that while the p75^{NTR} receptor played a role in the OVA-induced effects on pulmonary resistance, DEP-induced enhancement of Penh, and lung IL-4 levels in this model, the trkA receptor only influenced the DEP-induced enhancement in Penh and lung IL-4 levels. These findings highlight the probable diversity in function between the neurotrophins.

IgE plays a critical role in the generation of the asthmatic phenotype by activating the release of mast cell mediators that contribute to the bronchoconstriction and mucus production characteristic of asthma (Wills-Karp, 1999). DEP exposure in mice increases allergen-induced serum IgE levels (Whitekus *et al.*, 2002) and enhances IgE secretion from B cells in vitro (Tsien *et al.*, 1997). Neurotrophins may influence IgE and/or mast cell activity. The mast cell expresses the trkA receptor and IgE binding causes mast cell release of NGF (Kassel *et al.*, 2001; Xiang and Nilsson, 2000). In the present study, OVA-sensitized mice had increased OVA-specific IgE levels. DEP exposure and/or administration of either of the neurotrophin antibodies, however, did not influence circulating IgE levels. The lack of an enhancement in IgE due to DEP exposure may be reflective of the limited DEP exposures in the present study. These findings are consistent with studies that have suggested that neurotrophins may not influence IgE production (Lommatzsch *et al.*, 2003). A report by Path *et al.* (2002) showed that mice treated with anti-NGF antibodies had lower levels of Th2 cytokines and eosinophils but normal levels of serum IgE (Path *et al.*, 2002).

The pathologic features of asthma include mucus hypersecretion, and the infiltration of inflammatory cells (Holgate, 2000; Wills-Karp, 1999). DEP coexposure did not significantly enhance the pathologic features characteristic of asthma. Macrophages produce neurotrophins and express the trkA receptor (Ricci *et al.*, 2004). DEP exposure or OVA sensitization independently increased the number of macrophages in the lung. These increases were significantly attenuated by anti-trkA or anti-p75^{NTR} administration suggesting that normal macrophage airway infiltration is dependent on neurotrophins. When OVA-sensitized mice were coexposed to DEP, however, there was a decrease in the number of macrophages. The mechanism behind this decrease needs to be further investigated. OVA sensitization and DEP exposure, but not either alone, caused a significant increase in eosinophils relative to vehicle-sensitized air-exposed mice. There was no significant effect of anti-p75^{NTR} or anti-trkA instillation on eosinophil levels. OVA sensitization caused a significant increase in neutrophils that was unaffected by DEP coexposure and/or antibody administration. The absence of significant airway inflammation after allergen and DEP exposure may be due to the mild allergy protocol used and because the mice were only exposed once to DEP. This fact may also have contributed to the small change in lung resistance. Takano *et al.* (1998a) showed that repeated

DEP inhalation exposures were necessary to enhance airway allergy. Additional studies with a more robust allergic airways response after DEP exposure will help further delineate the role of neurotrophins in allergic airways disease.

The DEP-induced enhancement in ventilatory timing may have involved a nonimmune component of allergic airways disease, i.e., neurogenic inflammation. DEP exposure enhanced secretion of the neurokinin substance P and increased expression of the neurokinin receptor, NK-1, in the airways of rats (Wong *et al.*, 2003). Tian *et al.* (1997) showed that rats treated with a neurokinin-1 receptor antagonist had decreased airway responses to Mch. Neurotrophins activate the production of neurokinins such as substance P and neurokinin A (Vedder *et al.*, 1993). For example, NGF-induced enhancement of airway hyperresponsiveness in guinea pigs was inhibited by treatment with a neurokinin receptor antagonist (de Vries *et al.*, 1999). DEP exposure in OVA-sensitized mice in the present study may have increased neurotrophin activity, which in turn may have caused the production and release of neurokinins that mediate airflow changes. Anti-trkA and/or anti-p75^{NTR} treatment may have inhibited the DEP-induced enhancement in airflow obstruction by inhibiting the neurotrophin-induced activation of neurokinin production in the upper airways. The link between DEP exposure, neurotrophins, and neurokinins will be further investigated in future studies by the assessment of neurokinin and neurotrophin production via immunohistochemistry of the upper airways and nasal lavage.

The results of this study demonstrate that the enhancement of allergic airways responses by DEP exposure is partly dependent on neurotrophins in mice. Neurotrophins may play a similar role in humans. A better grasp of the direct and indirect influences of particulates on neurotrophin function will lead to a better appreciation of the effects of diesel particulates in the exacerbation of asthma. Therapeutic interventions may also be developed that target some component of neurotrophin signaling and thus may lead to an amelioration of asthma symptoms worsened by particulate exposure.

REFERENCES

- Bates, J., Irvin, C., Brusasco, V., Drazen, J., Fredberg, J., Loring, S., Eidelman, D., Ludwig, M., Macklem, P., Martin, J., *et al.* (2004). The use and misuse of Penh in animal models of lung disease. *Am. J. Respir. Cell Mol. Biol.* **31**, 373–374.
- Becker, S., Soukup, J. M., and Gallagher, J. E. (2002). Differential particulate air pollution induced oxidant stress in human granulocytes, monocytes and alveolar macrophages. *Toxicol. In Vitro* **16**, 209–218.
- Braun, A., Lommatzsch, M., Mannsfeldt, A., Neuhaus-Steinmetz, U., Fischer, A., Schnoy, N., Lewin, G. R., and Renz, H. (1999). Cellular sources of enhanced brain-derived neurotrophic factor production in a mouse model of allergic inflammation. *Am. J. Respir. Cell Mol. Biol.* **21**, 537–546.
- de Vries, A., Dessing, M. C., Engels, F., Henricks, P. A., and Nijkamp, F. P. (1999). Nerve growth factor induces a neurokinin-1 receptor-mediated airway hyperresponsiveness in guinea pigs. *Am. J. Respir. Crit. Care Med.* **159**, 1541–1544.

- Diaz-Sanchez, D., Tsien, A., Fleming, J., and Saxon, A. (1997). Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human *in vivo* nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. *J. Immunol.* **158**, 2406–2413.
- Dong, C. C., Yin, X. J., Ma, J. Y., Millechia, L., Wu, Z. X., Barger, M. W., Roberts, J. R., Antonini, J. M., Dey, R. D., and Ma, J. K. (2005). Effect of diesel exhaust particles on allergic reactions and airway responsiveness in ovalbumin-sensitized brown Norway rats. *Toxicol. Sci.* **88**, 202–212.
- Farraj, A. K., Haykal-Coates, N., Ledbetter, A. D., Evansky, P. A., and Gavett, S. H. (2006). Inhibition of pan neurotrophin receptor p75 attenuates diesel particulate-induced enhancement of allergic airway responses in C57/Bl6J Mice. *Inhal. Toxicol.* **18**, 483–491.
- Finotto, S., De Sanctis, G. T., Lehr, H. A., Herz, U., Buerke, M., Schipp, M., Bartsch, B., Atreya, R., Schmitt, E., Galle, P. R., *et al.* (2001). Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. *J. Exp. Med.* **193**, 1247–1260.
- Frew, A. J. (1996). The immunology of respiratory allergies. *Toxicol. Lett.* **86**, 65–72.
- Gavett, S. H., Haykal-Coates, N., Highfill, J. W., Ledbetter, A. D., Chen, L. C., Cohen, M. D., Harkema, J. R., Wagner, J. G., and Costa, D. L. (2003). World Trade Center fine particulate matter causes respiratory tract hyperresponsiveness in mice. *Environ. Health Perspect.* **111**, 981–991.
- Glaab, T., Hoymann, H. G., Hecht, M., Korolewitz, R., Tschernig, T., Hohlfeld, J. M., Krug, N., and Braun, A. (2003). Effect of anti-nerve growth factor on early and late airway responses in allergic rats. *Allergy* **58**, 900–904.
- Gomes, R. F., Shen, X., Ramchandani, R., Tepper, R. S., and Bates, J. H. (2000). Comparative respiratory system mechanics in rodents. *J. Appl. Physiol.* **89**, 908–916.
- Hamelmann, E., Schwarze, J., Takeda, K., Oshiba, A., Larsen, G. L., Irvin, C. G., and Gelfand, E. W. (1997). Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med.* **156**, 766–775.
- Hantos, Z., Daroczy B., Suki, B., Nagy, S., and Fredberg, J. J. (1992). Input impedance and peripheral inhomogeneity of dog lungs. *J. Appl. Physiol.* **72**, 168–178.
- Holgate, S. T. (2000). Inflammatory and structural changes in the airways of patients with asthma. *Respir. Med.* **94**(Suppl. D), S3–S6.
- Kassel, O., de Blay, F., Duvernelle, C., Olgart, C., Israel-Biet, D., Krieger, P., Moreau, L., Muller, C., Pauli, G., and Frossard, N. (2001). Local increase in the number of mast cells and expression of nerve growth factor in the bronchus of asthmatic patients after repeated inhalation of allergen at low-dose. *Clin. Exp. Allergy* **31**, 1432–1440.
- Kerzel, S., Path, G., Nockher, W. A., Quarcoo, D., Raap, U., Groneberg, D. A., Dinh, Q. T., Fischer, A., Braun, A., and Renz, H. (2003). Pan-neurotrophin receptor p75 contributes to neuronal hyperreactivity and airway inflammation in a murine model of experimental asthma. *Am. J. Respir. Cell Mol. Biol.* **28**, 170–178.
- Kimata, H. (2004). Exposure to road traffic enhances allergic skin wheal responses and increases plasma neuropeptides and neurotrophins in patients with atopic eczema/dermatitis syndrome. *Int. J. Hyg. Environ. Health* **207**, 45–49.
- Krane, C. M., Fortner, C. N., Hand, A. R., McGraw, D. W., Lorenz, J. N., Wert, S. E., Towne, J. E., Paul, R. J., Whitsett, J. A., and Menon, A. G. (2001). Aquaporin 5-deficient mouse lungs are hyperresponsive to cholinergic stimulation. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14114–14119.
- Ledbetter, A., Killough, P., and Hudson, G. (1998). A low-sample consumption dry-particulate aerosol generator for use in nose-inhalation exposure. *Inhal. Toxicol.* **10**, 239–251.
- Levitan, I., and Kaczmarek, L. (1997). The neuron: Cell and molecular biology. *Growth, Survival, and Differentiation of Neurons*, 2nd ed., pp. 357–368. Oxford University Press, New York.
- Lommatzsch, M., Braun, A., and Renz, H. (2003). Neurotrophins in allergic airway dysfunction: What the mouse model is teaching us. *Ann. N. Y. Acad. Sci.* **992**, 241–249.
- Lutchen, K. R., Hantos, Z., Petak, F., Adamicza, A., and Suki, B. (1996). Airway inhomogeneities contribute to apparent lung tissue mechanics during constriction. *J. Appl. Physiol.* **80**, 1841–1849.
- Nassenstein, C., Braun, A., Erpenbeck, V. J., Lommatzsch, M., Schmidt, S., Krug, N., Luttmann, W., Renz, H., and Virchow, J. C., Jr. (2003). The neurotrophins nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4 are survival and activation factors for eosinophils in patients with allergic bronchial asthma. *J. Exp. Med.* **198**, 455–467.
- Nightingale, J. A., Maggs, R., Cullinan, P., Donnelly, L. E., Rogers, D. F., Kinnersley, R., Chung, K. F., Barnes, P. J., Ashmore, M., and Newman-Taylor, A. (2000). Airway inflammation after controlled exposure to diesel exhaust particulates. *Am. J. Respir. Crit. Care Med.* **162**, 161–166.
- Nordenhall, C., Puorazar, J., Ledin, M. C., Levin, J. O., Sandstrom, T., and Adelroth, E. (2001). Diesel exhaust enhances airway responsiveness in asthmatic subjects. *Eur. Respir. J.* **17**, 909–915.
- Olgart Hoglund, C., de Blay, F., Oster, J. P., Duvernelle, C., Kassel, O., Pauli, G., and Frossard, N. (2002). Nerve growth factor levels and localisation in human asthmatic bronchi. *Eur. Respir. J.* **20**, 1110–1116.
- Ormstad, H. (2000). Suspended particulate matter in indoor air: Adjuvants and allergen carriers. *Toxicology* **152**, 53–68.
- Ostro, B., Chestnut, L., Vichit-Vadakan, N., and Laixuthai, A. (1999). The impact of particulate matter on daily mortality in Bangkok, Thailand. *J. Air Waste Manag. Assoc.* **49**(9 Spec No), 100–107.
- Pandya, R. J., Solomon, G., Kinner, A., and Balmes, J. R. (2002). Diesel exhaust and asthma: Hypotheses and molecular mechanisms of action. *Environ. Health Perspect.* **110**(Suppl. 1), 103–112.
- Path, G., Braun, A., Meents, N., Kerzel, S., Quarcoo, D., Raap, U., Hoyle, G. W., Nockher, W. A., and Renz, H. (2002). Augmentation of allergic early-phase reaction by nerve growth factor. *Am. J. Respir. Crit. Care Med.* **166**, 818–826.
- Reidl, M., and Diaz-Sanchez, D. (2005). Biology of diesel exhaust effects on respiratory function. *J. Allergy Clin. Immunol.* **115**, 221–228.
- Ricci, A., Felici, L., Mariotta, S., Mannino, F., Schmid, G., Terzano, C., Cardillo, G., Amenta, F., and Bronzetti, E. (2004). Neurotrophin and neurotrophin receptor protein expression in the human lung. *Am. J. Respir. Cell Mol. Biol.* **30**, 12–19.
- Siegel, P. D., Saxena, R. K., Saxena, Q. B., Ma, J. K., Ma, J. Y., Yin, X. J., Castronova, V., Al-Humadi, N., and Lewis, D. M. (2004). Effect of diesel exhaust particulate (DEP) on immune responses: Contributions of particulate versus organic soluble components. *J. Toxicol. Environ. Health A* **67**, 221–231.
- Steenenbergh, P. A., Withagen, C. E., Dormans, J. A., van Dalen, W. J., van Loveren, H., and Casee, F. R. (2003). Adjuvant activity of various diesel exhaust and ambient particles in two allergic models. *J. Toxicol. Environ. Health A* **66**, 1421–1439.
- Steiger, D., Hotchkiss, J., Bajaj, L., Harkema, J., and Basbaum, C. (1995). Concurrent increases in the storage and release of mucin-like molecules by rat airway epithelial cells in response to bacterial endotoxin. *Am. J. Respir. Cell Mol. Biol.* **12**, 307–314.
- Svartengren, M., Strand, V., Bylin, G., Jarup, L., and Pershagen, G. (2000). Short-term exposure to air pollution in a road tunnel enhances the asthmatic response to allergen. *Eur. Respir. J.* **15**, 716–724.
- Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T., and Dahlen, S. E. (2001). Health effects of diesel exhaust emissions. *Eur. Respir. J.* **17**, 733–746.
- Takano, H., Ichinose, T., Miyabara, Y., Shibuya, T., Lim, H. B., Yoshikawa, T., and Sagai, M. (1998a). Inhalation of diesel exhaust enhances allergen-related

- eosinophil recruitment and airway hyperresponsiveness in mice. *Toxicol. Appl. Pharmacol.* **150**, 328–337.
- Takano, H., Ichinose, T., Miyabara, Y., Yoshikawa, T., and Sagai, M. (1998b). Diesel exhaust particles enhance airway responsiveness following allergen exposure in mice. *Immunopharmacol. Immunotoxicol.* **20**, 329–336.
- Tian, J., Wei, E. Q., Chen, J. S., and Zhang, W. P. (1997). Effect of SR-140333, a neurokinin-1 receptor antagonist, on airway reactivity to methacholine in sedated rats. *Zhongguo Yao Li Xue Bao* **18**, 485–488.
- Tsien, A., Diaz-Sanchez, D., Ma, J., and Saxon, A. (1997). The organic component of diesel exhaust particles and phenanthrene, a major polycyclic aromatic hydrocarbon constituent, enhances IgE production by IgE-secreting EBV-transformed human B cells in vitro. *Toxicol. Appl. Pharmacol.* **142**, 256–263.
- Vedder, H., Affolter, H. U., and Otten, U. (1993). Nerve growth factor (NGF) regulates tachykinin gene expression and biosynthesis in rat sensory neurons during early postnatal development. *Neuropeptides* **24**, 351–357.
- Virchow, J. C., Julius, P., Lommatzsch, M., Luttmann, W., Renz, H., and Braun, A. (1998). Neurotrophins are increased in bronchoalveolar lavage fluid after segmental allergen provocation. *Am. J. Respir. Crit. Care Med.* **158**, 2002–2005.
- Whitekus, M. J., Li, N., Zhang, M., Wang, M., Horwitz, M. A., Nelson, S. K., Horwitz, L. D., Brechun, N., Diaz-Sanchez, D., and Nel, A. E. (2002). Thiol antioxidants inhibit the adjuvant effects of aerosolized diesel exhaust particles in a murine model for ovalbumin sensitization. *J. Immunol.* **168**, 2560–2567.
- Wills-Karp, M. (1999). Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu. Rev. Immunol.* **17**, 255–281.
- Wong, S. S., Sun, N. N., Keith, I., Kweon, C. B., Foster, D. E., Schauer, J. J., and Witten, M. L. (2003). Tachykinin substance P signaling involved in diesel exhaust-induced bronchopulmonary neurogenic inflammation in rats. *Arch. Toxicol.* **77**, 638–650.
- Xiang, Z., and Nilsson, G. (2000). IgE receptor-mediated release of nerve growth factor by mast cells. *Clin. Exp. Allergy* **30**, 1379–1386.
- Yssel, H., and Groux, H. (2000). Characterization of T cell subpopulations involved in the pathogenesis of asthma and allergic diseases. *Int. Arch. Allergy Immunol.* **121**, 10–18.