

Inhibition of Pan Neurotrophin Receptor p75 Attenuates Diesel Particulate-Induced Enhancement of Allergic Airway Responses in C57/B16J Mice

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Recent investigations have linked neurotrophins, including nerve growth factor (NGF), neurotrophin-3 (NT-3), and brain-derived neurotrophic factor (BDNF), to allergic airways diseases. Antibody blockade of NGF attenuates airway resistance in allergic mice. Diesel exhaust particle (DEP) exposure has been linked to asthma exacerbation in many cities with vehicular traffic congestion. We tested the hypothesis that DEP-induced enhancement of the hallmark features of allergic airway disease in a murine model is dependent on the function of the pan neurotrophin receptor p75. Ovalbumin (OVA)-sensitized C57B1/6J mice were intranasally instilled with an antibody against the p75 receptor or saline alone 1 h before OVA challenge. The mice were then exposed nose-only to the PM_{2.5} fraction of SRM2975 DEP or air alone for 5 h beginning 1 h after OVA challenge. Two days later, air-exposed OVA-allergic mice developed a small but insignificant increase in methacholine-induced airflow obstruction relative to air-exposed, vehicle-sensitized mice. DEP-exposed OVA-allergic mice had a significantly greater degree of airway obstruction than all other groups. Instillation of anti-p75 significantly attenuated the DEP-induced increase in airway obstruction in OVA-allergic mice to levels similar to non-sensitized mice. The DEP-induced exacerbation of allergic airway responses may, in part, be mediated by neurotrophins.

Neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) are important mediators of neuronal and immune function. Nerve functions of neurotrophins include growth and maintenance, neurotransmitter and neuropeptide production, and

excitability (Olgart Hoglund et al., 2002). Neurotrophins are expressed, along with their receptors, in many immune, structural, and nerve cells within the respiratory tract, including bronchial epithelium, pulmonary smooth muscle, mast cells, alveolar macrophages, eosinophils, T lymphocytes, ganglionic neurons, and fibroblasts (Ricci et al., 2004). Recent evidence suggests that neurotrophins are linked to the etiology of asthma. In one study, asthmatics had elevated levels of NGF in their lungs in comparison to healthy subjects (Virchow et al., 1998). In addition, NGF, BDNF, and NT-3 were necessary for normal eosinophil function in patients with allergic bronchial asthma (Nassenstein et al., 2003). Many animal studies of allergen-induced asthma have also demonstrated a link between neurotrophin levels and asthma. For example, BDNF expression in the respiratory epithelium was upregulated in allergic lungs of mice (Braun et al., 1999). Also, mice that overexpressed NGF had an enhanced response to allergen challenge (Path et al.,

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2002). In addition, functional silencing of the pan-neurotrophin receptor p75 in allergen-treated mice decreased airway inflammation (Kerzel et al., 2003).

NGF plays a role in the exacerbation of asthma. For example, Piedimonte established a role for NGF in the exacerbation of asthma by exposure to respiratory syncytial virus (Piedimonte, 2003). Epidemiologic and experimental studies indicate that exposure to diesel particulates exacerbates asthma (Sydbom et al., 2001). Structural and immune cells within the lung that are affected by diesel exhaust particles (DEP), including eosinophils, macrophages, lymphocytes, and the airway epithelium, also produce neurotrophins and/or express neurotrophin receptors. In addition, DEP exposure enhances many of the characteristic features of asthma that have been linked to NGF function, such as airway hyperresponsiveness and airway inflammation (Sydbom et al., 2001; Pandya et al., 2002). Neurotrophins have also been linked to DEP-induced exacerbation of other allergic diseases. In one study, exposure to road traffic enhanced allergen-induced increases in plasma levels of NGF in subjects with atopic dermatitis (Kimata, 2004). The importance of neurotrophins in DEP-induced exacerbation of asthma, however, is unknown.

The pan-neurotrophin receptor p75 is a low-affinity receptor for all neurotrophins (Ricci et al., 2004). The p75 receptor potentiates signaling through the tyrosine kinase (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 & Kaczmarek, 1997). We tested the hypothesis that DEP-induced enhancement of the hallmark features of allergic airways disease in a murine model is dependent on normal p75 function. Mice were made allergic to valbumin using an exposure regimen designed to elicit a mild allergic response. Ovalbumin-sensitized and challenged 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 receptor. Airway physiology, inflammation, and pathology were assessed to determine the importance of p75 function in DEP-induced exacerbation of allergic airways disease.

METHODS

Particulate Matter Sample

Mice that were treated with the particulate matter (PM) sample received Standard Reference Material 2975, which was collected from a filter from a diesel-powered industrial forklift (National Institute of Standards and Technology, Gaithersburg, MD).

Experimental Animals and Weight Randomization

Young adult (6-wk-old) male inbred C57/B16 mice were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were housed in plastic cages on beta-chip bedding in groups of 3 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 (9 mice per group).

Experimental Design and Treatment

Figure 1 depicts the exposure regimen used for mouse exposure. On day 0, mice were immunized via an intraperitoneal injection of 20 μ g ovalbumin in an aluminum hydroxide gel vehicle (Sigma Chemical, St. Louis, MO) or vehicle alone. Two weeks later, the mice were intranasally instilled with 50 μ l of rabbit anti-mouse p75 neurotrophin receptor polyclonal antibody (Chemicon, Temecula, CA; diluted 1:50 in sterile saline), as previously described (Kerzel et al., 2003) or saline alone. One hour later, the mice were challenged with a 1% ovalbumin aerosol for 1 h. One hour after ovalbumin challenge, the mice underwent a 5-h nose-only exposure to the NIST SRM-2975 diesel exhaust particulates or air.

A DEP concentration of 0.87 mg/m³ was selected as the exposure concentration because it optimally enhanced the allergic airways response, particularly airflow obstruction, as determined in a pilot study. Mice were exposed to DEP or filtered air in two separate nose-only inhalation exposure chambers. We conducted the exposures for 5 h in 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 particle charge neutralizer and a 2.5- μ m 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 (45 mm diameter with 1- μ m pore size; WVR Scientific, West Chester, PA), and real-time PM concentration was estimated with an aerosol monitor (Dust Track; TSI, Inc., St. Paul, MN) on the chamber

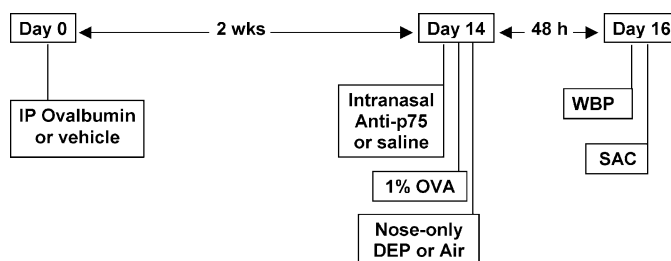


FIG. 1. Timeline for the exposure regimen for C57/B16J mice. Mice were sensitized with an intraperitoneal (ip) ovalbumin injection. Two weeks later, the mice were intranasally instilled with 50 μ l of anti-p75 and then challenged with a 1% ovalbumin aerosol (OVA) 1 h later. One hour after challenge, the mice were exposed nose-only to NIST SRM-2975 at a concentration of 0.87 mg/m³ or air for 5 h. Two days later, whole-body plethysmography (WBP) was performed followed by sacrifice (SAC).

exhaust. The particle size was determined gravimetrically using a Mercer Cascade Impactor (Intox Products, Albuquerque, NM).

In Vivo Airway Responses

We measured respiratory responsiveness to increasing aerosol concentrations of the airway smooth muscle agonist methacholine (MCH) in unanesthetized, unrestrained mice in a 12-chamber whole-body plethysmograph system (Buxco Electronics, Wilmington, NC) 48 h after DEP exposure. The pressure signal was mathematically converted into a unitless value called enhanced pause (Penh). Penh was used as an index of airflow obstruction as previously described (Hamelmann et al., 1997; Gavett et al., 2003). Mice with obstructed airflow have a higher Penh value at a given MCH concentration than normal mice. 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.

Bronchoalveolar Lavage

Mice were deeply anesthetized with urethane (1.5 g/kg ip). 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 Hanks 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 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 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 Th2 cytokines interleukin (IL)-4, IL-5, and IL-13 were carried out on an aliquot of BAL supernatant using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN). The limit of detection for these assays was the lowest standard concentration used in each assay. Samples with values less than the limit of detection were assigned the lowest standard concentration.

Histopathology

After removal of the right lung lobes, 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 has been described previously in detail (Steiger et al., 1995). Fixed lungs were processed to paraffin blocks, sectioned at an approximate thickness of $5 \mu\text{m}$, placed on glass slides, and stained with hematoxylin and eosin. The head of each mouse was excised from the carcass, and the eyes, skin, skeletal muscle, and lower jaw were then removed. In preparation for histopathologic analysis, the heads were immersed in 4% paraformaldehyde for 24 h. After fixation, the heads were decalcified in 13% formic acid for 7 days and then rinsed in tap water for at least 4 h. The nasal cavity of each mouse was transversely sectioned at three specific anatomic locations according to a modified method of Young (1981). The most proximal nasal section was taken immediately posterior to the upper incisor teeth (proximal, T1); the middle section was taken at the level of the incisive papilla of the hard palate (middle, T2); and the most distal nasal section was taken at the level of the second palatal ridge (distal, T3). These tissue blocks were embedded in paraffin, sectioned at a thickness of $5 \mu\text{m}$, and then stained with hematoxylin and eosin for light microscopic examination.

Serum IgE

Ovalbumin-specific serum immunoglobulin E (IgE) was measured using a 96-well Nunc ELISA plate (Nalge Nunc International, Rochester, NY) that was coated with 0.5 mg/ml ovalbumin (Sigma-Aldrich, St. Louis, MO) 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-ovalbumin mouse IgE standards (Serotec, Raleigh, NC) 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 at $2 \mu\text{g/ml}$ and allowed to incubate at 25°C for 1 h. After washing, $1.5 \mu\text{g/ml}$ of streptavidin peroxidase (Pharmingen, San Diego, CA) was added followed by incubation at 25°C for 1 h. After washing, tetramethylbenzidine (TMB) substrate (Alpha Diagnostic International, San Antonio, TX) was added. After color development, the reaction was terminated by the addition of H_2SO_4 (Alpha Diagnostic International, San Antonio, TX). 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, Sunnyvale, CA).

Statistical Analysis

All statistical analyses were done using SAS procedures (Cary, NC). There were two types of responses collected: (1) Penh responses, recorded repeatedly for each animal as average Penh for various concentrations of MCH, and (2) individual measurements measured once (differentials, ELISA data) for each animal as a univariate variable. Repeated-measures analysis of variance (ANOVA) was used for the Penh responses. One-way ANOVA was used to assess the individual measurements. The ANOVA in both cases was followed by Tukey's multiple-range

comparison test. Differences were considered significant if p was less than .05.

RESULTS

Airway Physiology

There were no significant differences in average Penh between any of the treatment groups in the vehicle controls at all MCH concentrations (Figure 2A). There were no significant

differences in any of the treatment groups in ovalbumin-sensitized mice at baseline, 0, 16, or 32 mg/ml of MCH. At 64 mg/ml MCH, ovalbumin-sensitized mice had a 22% increase in average Penh compared to vehicle-sensitized mice (vehicle-saline-air in Figure 2A vs. ovalbumin-saline-air in Figure 2B). Also at 64 mg/ml MCH, diesel-particulate-exposed ovalbumin-sensitized mice had a 68% increase in average Penh that was statistically significant relative to the air-exposed, vehicle-sensitized mice (vehicle-saline-air in Figure 2A vs. ovalbumin-saline-DEP in Figure 2B; $p < .05$). Intranasal instillation of anti-p75 completely inhibited the DEP-induced increase in average Penh in ovalbumin-sensitized mice to levels similar to air-exposed, vehicle-sensitized mice (ovalbumin-saline-DEP in Figure 2B vs. ovalbumin-anti-p75-DEP in Figure 2B; $p < .05$).

Airway Pathology

Mice that were sensitized and challenged with ovalbumin had small increases in the numbers of mucus-secreting cells in the epithelium lining the airway lumen. This epithelial change was accompanied by a mild influx of inflammatory cells that consisted of eosinophils and mononuclear cells, including lymphocytes (data not shown). DEP coexposure in ovalbumin-sensitized mice increased the number of mucus secreting cells and the severity of the inflammatory infiltrate in only one of the nine mice. There were no effects of intranasal anti-p75 administration. Ovalbumin and/or DEP exposure did not result in any lesions in the nasal airway.

Cells in Bronchoalveolar Lavage Fluid

DEP exposure in vehicle-sensitized mice significantly enhanced the number of lavaged macrophages by 161% relative to air-exposed vehicle-sensitized mice (Figure 3A; $p < .05$). Anti-p75 administration significantly suppressed the DEP-induced increase in macrophages in vehicle-sensitized mice ($p < .05$). Ovalbumin-sensitized mice that were exposed to air had a 69% increase in the number of macrophages in the BAL fluid relative to the air-exposed vehicle-sensitized mice (Figure 3B vs. Figure 3A). There were no significant effects of DEP exposure or anti-p75 treatment on macrophage numbers in OVA-allergic mice (Figure 3B).

Ovalbumin sensitization in air-exposed mice caused a several hundredfold increase in the number of eosinophils in the lavage fluid relative to vehicle-sensitized air-exposed controls (10×10^4 cells in ovalbumin-saline-air group in Figure 3B vs. 0.01×10^4 cells in vehicle-saline-air group in Figure 3A; $p < .05$). There were no significant effects of DEP exposure or anti-p75 treatment on eosinophil numbers in ovalbumin-sensitized mice. There were also no significant effects of ovalbumin and/or DEP exposure on the number of lymphocytes or neutrophils in the lavage fluid.

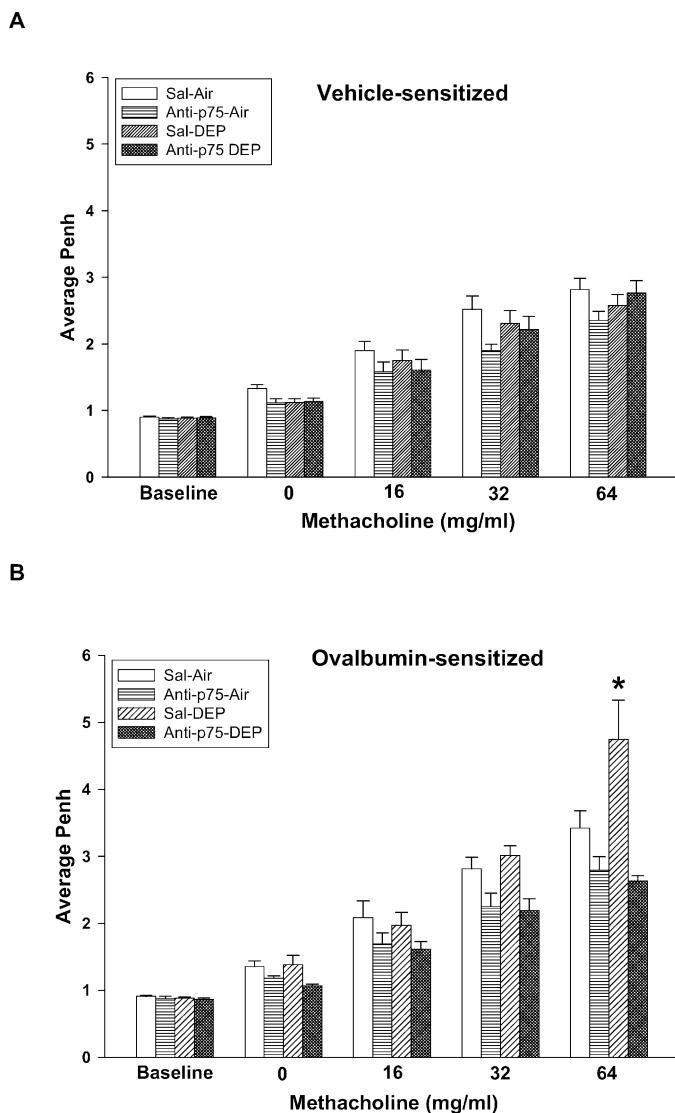


FIG. 2. Average Penh values from C57/Bl6J exposed to increasing concentrations of methacholine. (A) Vehicle or (B) ovalbumin (Ova)-sensitized mice were intranasally instilled with anti-p75 or saline (Sal) and then exposed nose-only to NIST-SRM 2975 DEP or air. Average Penh values were determined 48 h after DEP exposure. Symbols represent average Penh values $\pm$ the standard error ($n = 9$ /group). Asterisk indicates significantly greater than all other groups at 64 mg/ml MCH ($p < .05$).

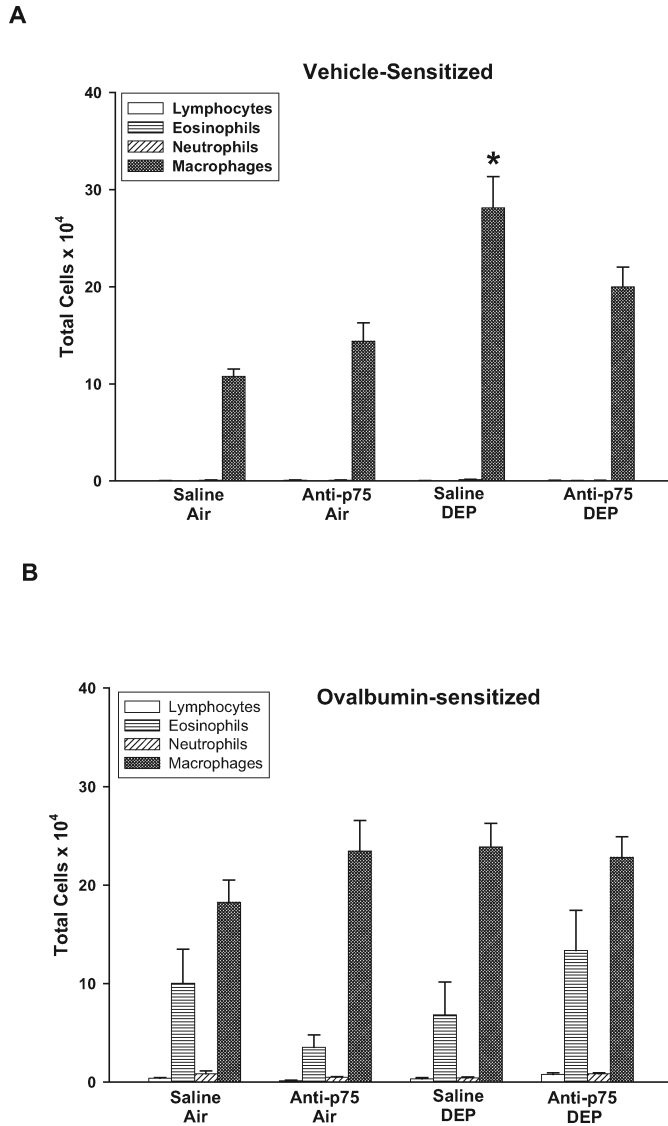


FIG. 3. Cells in bronchoalveolar lavage fluid (BALF) from C57/Bl6J mice. (A) Vehicle or (B) ovalbumin (Ova)-sensitized mice were intranasally instilled with anti-p75 or saline (Sal) and then exposed nose-only to NIST-SRM 2975 DEP or air. Lavage fluid was collected 48 h after DEP exposure. Bars represent average cell number per milliliter lavage fluid $\pm$ the standard error ($n = 9/\text{group}$). Asterisk indicates significantly greater than all other vehicle-sensitized groups in the number of macrophages ($p < .05$).

Cytokines

Ovalbumin 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 (Figure 4). DEP exposure also did not enhance the levels of IL-4 protein in BALF. In addition, there was no significant effect of anti-p75 administration on IL-4 protein levels in the lavage fluid. Only

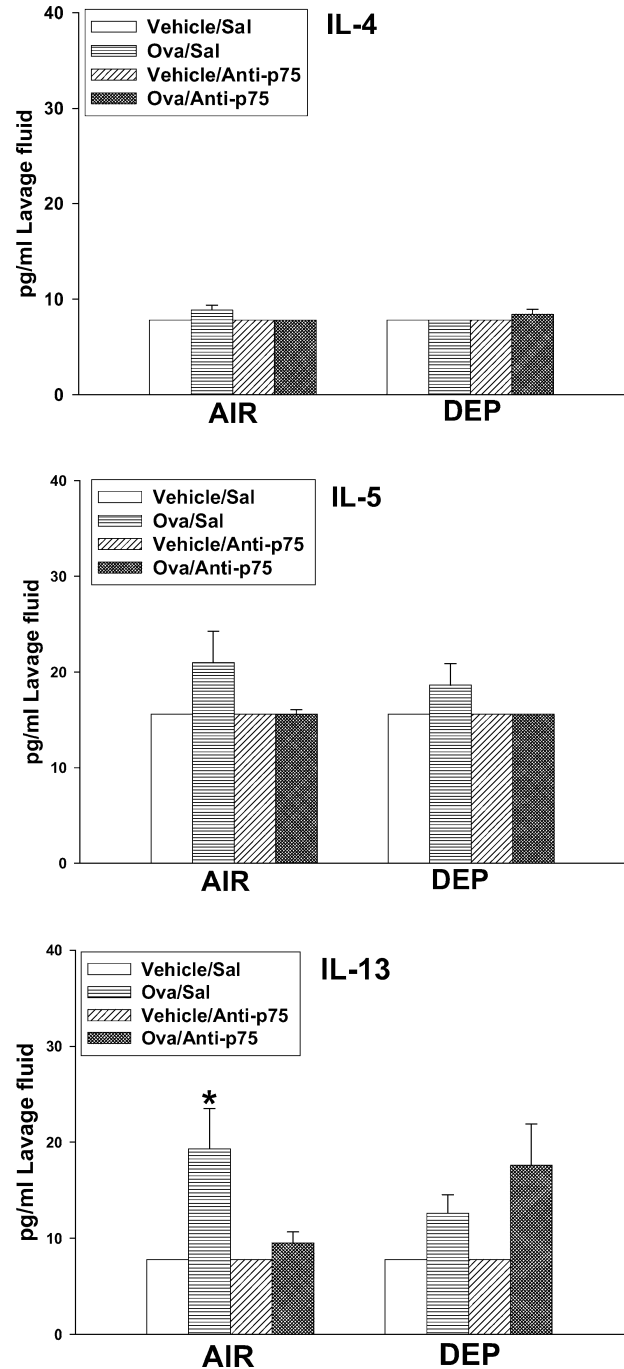


FIG. 4. IL-4, IL-5, or IL-13 protein levels (top, center, bottom, respectively) in bronchoalveolar lavage fluid (BALF) from C57/Bl6J. Vehicle or ovalbumin (Ova)-sensitized mice were intranasally instilled with anti-p75 or saline (Sal) and then exposed nose-only to NIST-SRM 2975 DEP or air. Lavage fluid was collected 48 h after DEP exposure. Bars represent average amounts of cytokine (pg/ml lavage fluid) $\pm$ the standard error ($n = 9/\text{group}$). 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. Asterisk indicates significantly greater than all vehicle-sensitized groups in the levels of IL-13 ($p < .05$).

the ovalbumin-sensitized groups had IL-4 readings above the limit of detection.

Ovalbumin sensitization in air-exposed mice caused a 34% increase in IL-5 protein levels in the BAL fluid relative to vehicle-sensitized, air-exposed mice, which was not statistically significant. Anti-p75 administration and/or DEP exposure had no significant effect on IL-5 levels. Only the ovalbumin-sensitized groups had IL-5 readings above the limit of detection.

Ovalbumin sensitization in air-exposed mice caused a 148% increase in IL-13 protein levels in the BAL fluid relative to vehicle-sensitized, air-exposed mice ($p < .05$). Anti-p75 administration slightly decreased the ovalbumin-induced increase in IL-13 in air-exposed mice, but this decrease was not statistically significant. There were no significant effects of DEP or anti-p75 treatment on IL-13 levels. Only the ovalbumin-sensitized groups had IL-5 readings above the limit of detection.

Serum IgE

Ovalbumin sensitization in the presence or absence of DEP and/or anti-p75 caused at least a threefold increase in ovalbumin-specific serum IgE levels relative to the respective vehicle controls in air-exposed mice (Figure 5; $p < .05$). There were no significant effects of DEP or anti-p75 treatment on serum IgE levels.

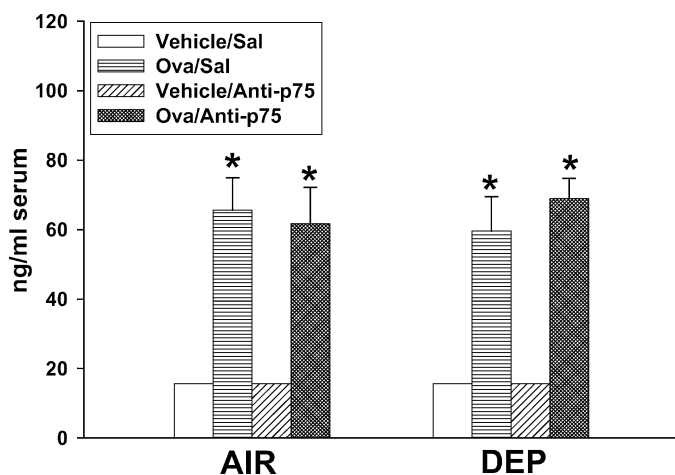


FIG. 5. Ovalbumin-specific serum IgE levels in C57/Bl6J mice. Vehicle or ovalbumin (Ova)-sensitized mice were intranasally instilled with anti-p75 or saline (Sal) and then exposed nose-only to NIST-SRM 2975 DEP or air. Serum was collected 48 h after DEP exposure. Bars represent average amounts of IgE (pg/ml serum) $\pm$ the standard error ($n = 9$ /group). The minimum amount detectable for this assay was 15.6 ng/ml. Asterisk indicates significantly greater than all vehicle-sensitized groups ($p < .05$).

DISCUSSION

This report demonstrates that neurotrophins are at least partly responsible for the exacerbation of airflow obstruction by DEP in a murine model of asthma. DEP-exposed mice that were made allergic to ovalbumin using a mild allergy protocol had a significantly greater degree of methacholine-induced airflow changes than air-exposed ovalbumin-allergic mice, as determined using whole-body plethysmography. Intra-airway administration of an antibody against the pan neurotrophin receptor p75 attenuated the DEP-induced increase in airflow obstruction in ovalbumin-allergic C57/Bl6 mice.

A characteristic feature of asthma is airway hyperresponsiveness. DEP exposure enhances airway hyperresponsiveness in asthmatics. For example, the increase in airway resistance and decrease in lung function in asthmatics exposed for 30 min to vehicular exhaust in a Stockholm tunnel were significantly associated with high PM_{2.5} levels (Svartengren et al., 2000). In addition, diesel particulate exposure in mice enhanced allergen-induced airway responsiveness (Takano et al., 1998). Neurotrophins have also 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). Penh is an indicator of changes in ventilatory timing. These changes include increases in expiratory time and peak expiratory flow and decreases in inspiratory time and peak inspiratory flow (Hamelmann et al., 1997). Although the meaning of Penh is controversial, and changes in this parameter could result from changes in breathing patterns distinct from changes in airflow obstruction (Bates et al., 2004), strong correlations between increased Penh and increased airway resistance or airflow obstruction are often found in direct comparisons of the techniques (Hamelmann et al., 1997; Krane et al., 2001; Finotto et al., 2001). Direct measurement of airway resistance in mice that have a tracheal canulae inserted will miss the contribution to airflow resistance made by the nasal passages that is accounted for by the Penh parameter. This is especially important in light of the fact that DEP, ovalbumin, and anti-p75 antibody were all administered nasally in this study. In the present study, we found that DEP exposure enhanced average Penh values in ovalbumin-allergic C57/Bl6 mice. The intranasal instillation of anti-p75 attenuated the DEP-induced increase in Penh to control levels. This finding suggests that neurotrophins may, in part, mediate the DEP-induced enhancement of airflow obstruction.

Allergen-induced asthma and rhinitis are mediated by Th2 cytokines including interleukin (IL)-4, IL-5, and IL-13. IL-4 causes isotype switching in B cells (Frew, 1996). IL-5 recruits and activates eosinophils (Yssel & Groux, 2000). IL-13 is also involved in isotype switching in B cells and increased mucus production in the airways (Shim et al., 2001). DEP exposure influences Th2 cytokine production. In one study, DEP exposure enhanced Th2 cytokine production in the airways of humans exposed to ragweed allergen (Diaz-Sanchez et al., 1997). Th2 cytokine expression is very likely influenced by neurotrophins.

T cells produce both NGF and BDNF and may express the trkA receptor (Braun et al., 1999; Ricci et al., 2004). In the present study, DEP exposure did not enhance the ovalbumin-induced increase in IL-4, IL-5, or IL-13. The lack of a DEP-induced enhancement in Th2 cytokines may in part be due to the fact that the mice were exposed only once to the particulates. Repeated DEP exposure, as described in other murine models, causes an enhancement in Th2 cytokine production and secretion (Whitekus et al., 2002; Miyabara et al., 1998). A modification of the exposure regimen used in this model to increase the number of DEP exposures may lead to a stronger allergic airways response, particularly an increase in Th2 cytokines.

IgE plays a critical role in the generation of the asthmatic phenotype by activating the release of mast-cell mediators that contribute to the vasodilation, bronchoconstriction, and mucus production characteristic of the asthmatic airway (Wills-Karp, 1999). DEP exposure in mice increases allergen-induced serum IgE levels (Whitekus et al., 2002). DEP has also been shown to enhance IgE secretion from B cells in vitro (Pandya et al., 2002). Neurotrophins may influence IgE and/or mast-cell activity. The binding of IgE to mast cells causes release of NGF (Xiang & Nilsson, 2000). The mast cell also expresses the NGF receptor trkA (Kassel et al., 2001). In the present study, ovalbumin sensitization and challenge increased ovalbumin-specific serum IgE levels. DEP exposure, however, did not enhance circulating IgE levels. The lack of a DEP-induced enhancement in IgE may again be due to the limited DEP exposure. Serum IgE levels were also not influenced by anti-p75 administration. These findings are consistent with other reports that have suggested that neurotrophins do not influence diesel particulate-induced enhancement of IgE production (Lommatzsch et al., 2003).

The pathologic features of asthma include mucus hypersecretion, plasma exudation, and the infiltration of inflammatory cells, including B cells, T cells, and eosinophils (Wills-Karp, 1999; Holgate, 2000). Ovalbumin sensitization caused a significant increase in both eosinophils and macrophages in the lavage fluid in air-exposed mice. DEP exposure in vehicle-sensitized mice caused a significant increase in the numbers of macrophages in the lavage fluid. This finding is consistent with other reports that have found that DEP exposure alone causes an infiltration of inflammatory cells including macrophages into the lung (Walters et al., 2001). Anti-p75 administration inhibited the DEP induced increase in macrophages in vehicle-sensitized mice. This inhibition may be due to direct effects of the antibody on macrophages. Macrophages express neurotrophin receptors and secrete NGF and BDNF (Ricci et al., 2004). DEP exposure in ovalbumin-sensitized mice, however, did not cause a significant enhancement in the number of macrophages or eosinophils in the lung. This may be due to the mild allergy protocol used and the fact that the mice were only exposed once to DEP. There is evidence that suggests that increasing the number of DEP exposures in this murine model may enhance the inflammatory-cell infiltrate (Whitekus et al., 2002; Miyabara et al., 1998).

These findings suggest that changes in ventilatory parameters are more sensitive to DEP exposure than pathologic changes. An explanation for the increase in airflow obstruction in the absence of airway inflammation includes the possible DEP-induced enhancement of a nonimmune component of allergic airways disease, that is, neurogenic inflammation. DEP exposure influences neurokinin activity. In a recent study, 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). Neurokinins play a significant role in the alteration of airway physiology. For example, investigators determined that rats treated with a neurokinin-1 receptor antagonist had decreased airway responses to the airway smooth muscle agonist methacholine (Tian et al., 1997). One of the many functions of neurotrophins is to activate the production of neurokinins such as substance P and neurokinin A (Vedder et al., 1993). NGF-induced enhancement of airway hyperresponsiveness was inhibited by treatment with a neurokinin receptor antagonist in a study using guinea pigs (de Vries et al., 1999). DEP exposure in ovalbumin-sensitized mice in the present study may have caused an increase in neurotrophin activity, which in turn may have caused the production and release of neurokinins that mediate airflow obstruction. Anti-p75 treatment may have inhibited the DEP-induced enhancement in airflow obstruction by inhibiting the neurotrophin-induced activation of neurokinin production. The establishment of a relationship between neurotrophins and neurokinins in DEP-induced exacerbation of asthma requires further study.

The results of this study suggest that DEP enhancement of allergic airway responses in mice is partly mediated by neurotrophins. The possibility exists that asthma exacerbation in humans caused by exposure to diesel particulates is to some degree mediated by neurotrophins. An increased understanding 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 allergic airways diseases.

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