

This article was downloaded by:[Advanced Institute of Sanita]
[Advanced Institute of Sanita]

On: 16 May 2007

Access Details: [subscription number 769790701]

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954

Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Toxicology and Environmental Health, Part A Current Issues

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title-content=t713667303>

Increased Neurotrophin Production in a *Penicillium chrysogenum*-Induced Allergic Asthma Model in Mice

To cite this Article: Chung, Yong Joo, Farraj, Aimen, Coates, Najwa Haykal, Gavett, Stephen H. and Ward, Marsha D. W. , 'Increased Neurotrophin Production in a *Penicillium chrysogenum*-Induced Allergic Asthma Model in Mice', Journal of Toxicology and Environmental Health, Part A, 70:12, 1020 - 1026

To link to this article: DOI: 10.1080/15287390601172023

URL: <http://dx.doi.org/10.1080/15287390601172023>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article maybe used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

© Taylor and Francis 2007

Increased Neurotrophin Production in a *Penicillium chrysogenum*-Induced Allergic Asthma Model in Mice

Yong Joo Chung, Aimen Farraj, Najwa Haykal Coates, Stephen H. Gavett, and
 Marsha D. W. Ward

National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection
 Agency, Research Triangle Park, North Carolina, USA

Neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin (NT)-3, have been implicated in the pathogenesis of many features and symptoms of asthma. The role of neurotrophins in fungal allergic asthma, however, is unknown. Repeated pulmonary challenge with *Penicillium chrysogenum* extract (PCE) induces dose-dependent allergic asthma-like responses in mice. The aim of this study was to investigate whether neurotrophins are involved in the PCE-induced allergic airway response in mice. Mice were exposed to 10, 20, 50, or 70 µg PCE by involuntary aspiration 4 times over 1 mo. Bronchial alveolar lavage fluid (BALF) was collected immediately before and after the final exposure. The levels of NGF, NT-3, and NT-4 were determined by enzyme-linked immunosorbent assay (ELISA). The lungs were fixed and processed for immunohistochemical examination of NGF production. PCE-exposed mice had dose-dependent increases in NGF, NT-3, and NT-4 in both BALF and sera. Exposures to PCE produced elevation in positive immunohistochemical staining for NGF in the airway epithelium and smooth muscle cells, in addition to infiltrated cells such as mononuclear cells, eosinophils, and macrophages. Taken together, this is the first study to link fungal allergic asthma in an experimental model with enhanced production of neurotrophins in the airways, and suggests that neurotrophins may play a role in the etiology of mold-induced asthma in humans.

Neurotrophins (NTs) such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin (NT)-3 and NT-4, are structurally and functionally related proteins that play important roles in the survival, differentiation,

and maintenance of neuronal cells (Lewin & Barde, 1996). Their known biological effects are mediated by binding either to the high-affinity specific receptors trkA, trkB, and trkC or to the low-affinity receptor p75. NTs and their receptors have been typically studied for their roles in the peripheral and central nervous system functions. Recent discoveries, however, have pointed to a role for neurotrophins in inflammatory diseases including asthma (Braun et al., 1998; Kassel et al., 2001; Nassenstein et al., 2003, 2004; Noga et al., 2002). For example, asthmatics had increased levels of neurotrophins in their lungs (Virchow et al., 1998). The source of elevated NGF in the lungs of asthmatics is attributed to infiltrating inflammatory cells as well as structural cells in the respiratory tract (Olgart Hoglund et al., 2002). Many cells within the respiratory tract secrete neurotrophins, including Th2 type T-cells (Bonini et al., 1999), eosinophils (Noga et al., 2003), mast cells (Xiang & Nilsson, 2000) and structural cells such as airway epithelial cells (Fox et al., 2001), lung fibroblasts (Olgart & Frossard, 2001), and smooth muscle (Freund et al., 2002). In addition, Nassenstein et al. (2004) described the involvement of NTs in airway inflammation, airway hyperresponsiveness, and airway remodeling, which are all clinical characteristics of allergic asthma. Experimental studies in animals have also linked NGF and other neurotrophins to allergic inflammation and asthma (Braun et al., 2001; De Vries et al., 2002; Path et al., 2002). Furthermore, it was suggested that NTs are mediators between immune and neuronal responses that are characteristic of asthma (Frossard et al., 2004; Piedimonte, 2003). Elevated NGF acts in an autocrine and/or paracrine fashion to regulate recruitment and activation of effector cells in allergic bronchial asthma (Nassenstein et al., 2003; Kassel et al., 2001; Bonini et al., 1999).

Molds are ubiquitous in the environment, and exposure to molds contributes to various human diseases including asthma. The *Penicillium* species of mold, especially *P. chrysogenum*, are common indoor contaminants and are associated with asthma (Chou et al., 2003). Several investigators demonstrated that exposure of animals to viable conidia of *P. chrysogenum*

Received 26 July 2006; accepted 12 October 2006.

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

Address correspondence to Yong Joo Chung, U.S. EPA, 109 T W Alexander Drive, MD 143-01, Research Triangle Park, NC 27711, USA. E-mail: chung.yongjoo@epa.gov

induced allergic asthma responses, including increased serum immunoglobulin (Ig) E and airway eosinophilia (Cooley et al., 2000; Schwab et al., 2003). Recently, our group demonstrated that multiple exposures to *P. chrysogenum* extract (PCE) induced dose-dependent responses characteristic of allergic asthma such as airway eosinophilia, increased specific IgE, and airway hyperresponsiveness (Chung et al., 2005). Furthermore, an influx of inflammatory cells including macrophages, monocytes, and lymphocytes was observed in peribronchiolar and perivascular regions of the lungs of PCE-exposed mice.

Mold directly and indirectly affects many cell types within the respiratory tract. For example, intratracheal exposures to *Stachybotrys chartarum* or *Aspergillus fumigatus* spores induce the release of cytokines and chemokines from macrophages, T-cells, and/or epithelial cells (Rand et al., 2006; Hudson et al., 2005; Rivera et al., 2005; Schelenz et al., 1999). The cell types within the airways that are affected by mold, including eosinophils, macrophages, lymphocytes, and the airway epithelium, produce neurotrophins and/or express neurotrophin receptors. Further, mold exposure enhances features of asthma that are linked to NGF function, such as airway hyperresponsiveness and airway inflammation. The role of neurotrophins in mold-induced asthma, however, is unknown. The main purpose of this study was to determine whether mold exposure in a murine model of PCE-induced asthma affects neurotrophin levels in the lung and circulation. In addition, the specific cell types that produce NGF in the lung after mold exposure were determined using immunohistochemistry.

MATERIALS AND METHODS

Animals

Fifty-day-old female BALB/c mice weighing about 18 g (Charles River, Raleigh, NC) were group housed in polycarbonate cages with cellulose bedding in an environmentally controlled, American Association for Accreditation of Laboratory Animal Care-accredited vivarium. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of NHEERL, U.S. EPA. Mice were maintained on a 12-h light/dark cycle and allowed access to food (Purina Rodent Lab Chow, St. Louis, MO) and water ad libitum. One day after arrival, a microchip (Labtrac, Avid Identification Systems, Inc., Norco, CA) was subcutaneously implanted to track individual animals. Mice were allowed to acclimate 1 wk prior to the start of the experiment.

Fungal Antigen Preparation

Penicillium chrysogenum was isolated from BALF from a patient in Ohio. Subcultured *P. chrysogenum* was grown on a nylon filter resting on plates of potato dextrose agar at 23°C for 6 wk. This mass was extracted both by grinding with a sterile mortar and pestle for approximately 5 min and by homogenizing with a Polytron (Kinematica, Basel, Switzerland) for 2 min

in a total of 15 volumes (by weight) of Hanks balanced salt solution (HBSS; Gibco BRL, Life Technologies, Inc., Rockville, MD) plus 0.05% (v/v) Tween 80 (Fisher Scientific, Pittsburgh, PA). The resulting suspension was stirred overnight at 4°C, and then centrifuged at 12,500 × g for 1 h at 4°C. The supernatant was decanted, and then adjusted to pH 6 with HCl. This filtrate was concentrated with a stirred-cell concentrator (Amicon, Inc., Beverly, MA) using YM3 membranes (Millipore Corp. Bedford, MA; molecular weight <3000 Da cutoff) and filter sterilized with a 0.2-μm syringe filter. The crude antigen preparation (PCE) was assayed for total protein concentration using Pierce BCA protein assay kit (Pierce, Rockford, IL) according to the manufacturer's procedures. Then, PCE was stored at -80°C until use. The endotoxin level within the extract was measured using a *Limulus* amebocyte lysate test kit (BioWhittaker, Walkersville, MD); the level was 0.45 EU/ml.

Experimental Design

It is difficult to correlate the doses used in this study to human exposure. However, the number of viable conidia used in other animal studies is compared to the doses used in this study in terms of induction of allergic responses. Schwab et al. (2003) and Cooley et al. (2000) reported that exposure to low levels (10² viable conidia) of *P. chrysogenum* did not induce significant lung inflammation or increased serum immunoglobulins (Schwab et al., 2003) while exposure to 10⁴ *P. chrysogenum* conidia induced type 2 T-helper-cell-mediated inflammatory responses as well as allergic responses (Cooley et al., 2000). These studies support a threshold level for induction of allergic responses.

The dosing regimen in this study was described previously (Chung et al., 2005). Four groups of 15 mice each were exposed to 10, 20, 50, or 70 μg PCE in 50 μl total volume by involuntary aspiration (IA) 4× over a 4-wk period, as described in Ward et al. (2000). Briefly, mice were anesthetized by inhalation of a mixture of 3% isoflurane and 97% oxygen. After deposition of PCE in the oropharynx, mice inhaled the PCE when their noses were gently occluded with a fingertip. A group of control mice (vehicle control) was concurrently IA-exposed to HBSS alone. For a nonspecific inflammation control, a group of mice were IA-exposed to 3 doses of HBSS and a final single dose of PCE in 50 μl total volume. Serum and BALF were collected before d 0 (D0), and at d 1 (D1) and d 3 (D3) following the final exposure. The responses of these animals were compared to results obtained from HBSS control animals.

Bronchoalveolar Lavage and Blood Collection

Blood and BALF samples were collected as previously described in Ward et al. (1998). Briefly, mice were anesthetized with sodium pentobarbital and blood samples were collected by cardiac puncture. The blood was allowed to clot for 1–2 h at room temperature prior to serum separation by

centrifugation, and the serum was stored at -80°C until analysis. The lungs were lavaged twice with 1-ml aliquots of HBSS, and the BALF aliquots were pooled and stored on ice. The BALF was centrifuged at $100 \times g$ for 15 min at 4°C , and then the supernatant was decanted. Aliquots of BALF supernatant were stored at -80°C until analysis.

Neurotrophin Assays by ELISAs

All reagents and incubation periods were at room temperature and all volumes added were 100 μl unless otherwise noted. NGF, NT-3, NT-4, and BDNF enzyme-linked immunosorbent assays (ELISAs) were performed using NGF, NT-3, NT-4, and BDNF Emax immunoassay kits, respectively, according to the manufacturer's instructions (Promega Corp., Madison, WI). Briefly, coating antibody (anti-NGF, anti-NT-3, anti-NT-4, or anti-BDNF) in carbonate coating buffer, pH 9.7, was placed in flat bottom microtiter plates (Nunc International, Rochester, NY), and then the plates were sealed and incubated overnight at 4°C . Following blocking with 200 μl Block & Sample buffer, pH 7.6 (BSB), per well, either BALF samples or mouse serum and purified NGF, NT-3, NT-4, or BDNF standards were applied and allowed to incubate for 6 h (or for 2 h for BDNF assay). Biotinylated anti-NGF, anti-NT-3, anti-NT-4, or anti-BDNF antibodies in BSB were then added and allowed to incubate overnight at 4°C (or for 2 h at room temperature for BDNF assay). Streptavidin-horseradish peroxidase conjugate was applied and the plates were then incubated for 2.5 h (or 1 h for BDNF assay) at room temperature. One-Step Substrate Solution was added and incubated for at least 20 min. Reaction was stopped by adding 1 *N* hydrochloric acid. Optical density was read on a SpectraMax 340PC plate reader (Molecular Devices Corp., Menlo Park, CA) at a wavelength of 450 nm. Softmax Pro software (version 2.6.1, Molecular Devices Corp.) was used for data collection and conversion from optical density to neurotrophin concentrations. The limit of detection for these assays was 6.25 pg/ml (BALF) and 12.5 pg/ml (serum).

Immunohistochemistry

After bronchoalveolar lavage, the lungs were fixed in 10% buffered formalin acetate (Fisher Scientific, Fair Lawn, NJ). Lungs were embedded in paraffin, and sectioned along the main stem bronchus. Paraffin-embedded lung sections were placed in slide racks in an oven at 40°C for 30 min. The sections were then deparaffinized by incubating in a series of solutions including xylene, 100%, 95%, 80%, and 70% ethanol, and 0.1 *M* Tris-buffered saline, pH 7.2 (TBS). The deparaffinized sections were placed in peroxidase blocking reagent from DAKO (Dako Cytomation, Inc., Carpinteria, CA) for 30 min to suppress endogenous peroxidase activity. After rinsing in TBS, the sections were incubated in a blocking solution (10% [v/v] normal goat serum with 0.1% Triton X-100 in TBS) for 30 min at room temperature to block the nonspecific binding sites. The sections were then incubated with anti-mouse

NGF antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), diluted 1:100 in TBS containing 3% normal goat serum and 0.1% Triton X-100, for 2 h at room temperature. After washing in TBS (4×10 min), a dextran polymer backbone conjugated to secondary antibody and labeled with horseradish peroxidase (DAKO) was applied to the sections and incubated for 30 min at room temperature. Following the incubation, the slides were washed twice with TBS, and DAB+ substrate-chromogen solution was applied to the sections and incubated for 10 min at room temperature. The slides were washed four times with TBS followed by application of DAB (3,3-diaminobenzidine tetrahydrochloride) to the sections to visualize immunoreactivity. A couple of negative controls were tested for the effectiveness of the primary and the secondary antibodies. In the absence of the primary antibody (anti-NGF antibody) or the secondary antibody (anti-IgG-HRP), no NGF-positive cells were observed.

Statistics

The data were analyzed using analysis of variance with the SAS program (SAS Institute, Inc., Cary, NC). Pairwise comparisons were performed as subtests of the overall model. In cases of comparing a control group to other groups, Tukey's test was used. Significance was attributed to an effect or difference if the probability was less than .05. Adjustments in significance levels, for multiple comparisons, were made using a modified Bonferroni correction.

RESULTS

NGF Levels

NGF levels were significantly increased in the BALF of mice exposed to 50 μg PCE (PC50) and 70 μg PCE (PC70) at D0 and D1 compared to HBSS (vehicle) controls (Figure 1A). NGF levels in BALF of PC70 at D3 were also significantly increased. Mice exposed to 20 μg PCE (PC20) showed a significant elevation in BALF NGF levels at D3 compared to D0. Additionally, PC50 mice showed a significant rise in NGF levels at D1 compared to D0. A single exposure to PCE (HBPC) did not increase BALF NGF.

Serum NGF levels were significantly increased in mice exposed to PC70 (D0 and D1) and PC50 (D1 and D3) compared to HBSS controls (Figure 1B). The serum NGF level of PC50 mice was significantly higher at D3 than at D0. A single exposure to PCE (HBPC) did not increase serum NGF.

NT-4 Levels

BALF NT-4 levels were significantly increased in PC70 mice at D0, D1, and D3 and PC50 mice at D1 and D3 compared to HBSS controls (Figure 2A). Mice exposed to PC50 showed a significant elevation in BALF NT-4 levels at D1 compared to D0. A single exposure to PCE (HBPC) did not increase BALF NT-4.

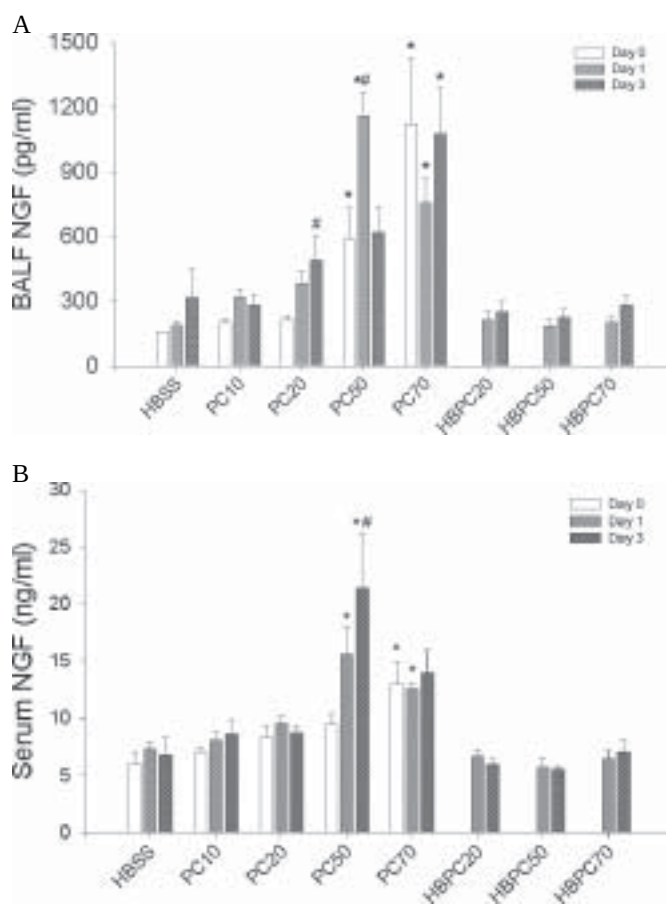


FIG. 1. NGF levels: (A) BALF NGF, (B) serum NGF. Mice were exposed 4 times to PCE and compared to naive (HBSS) mice at d 0, 1, and 3 after the final exposure. NGF levels were measured by ELISA. Asterisk indicates significant difference from HBSS control within time points ($p < .05$). #, Significant difference from d 0 control within treatment group ($p < .05$). Error bars represent standard error of the mean; $n = 4-5$. PC 10, 10 μ g *P. chrysogenum* extract (PCE); PC 20, 20 μ g PCE; PC 50, 50 μ g PCE; PC 70, 70 μ g PCE.

Serum NT-4 levels were significantly increased in mice exposed to PC50 at D0 and D1, and in mice exposed to PC70 at D3 (Fig 2B). A single exposure to PCE (HBPC) did not increase serum NT-4.

NT-3 Levels

NT-3 levels in BALF were significantly increased in PC70-exposed mice at D0, D1, and D3 and in PC50-exposed mice at D1 and D3 compared to HBSS controls (Figure 3A). In PC 50-exposed mice, BALF NT-3 levels were significantly elevated at D1 compared to D0. A single exposure to PCE (HBPC) did not increase BALF NT-3.

Serum NT-3 levels were significantly increased in PC50-exposed mice at D1 compared to HBSS controls (Figure 3B). A single exposure to PCE (HBPC) did not increase serum NT-3.

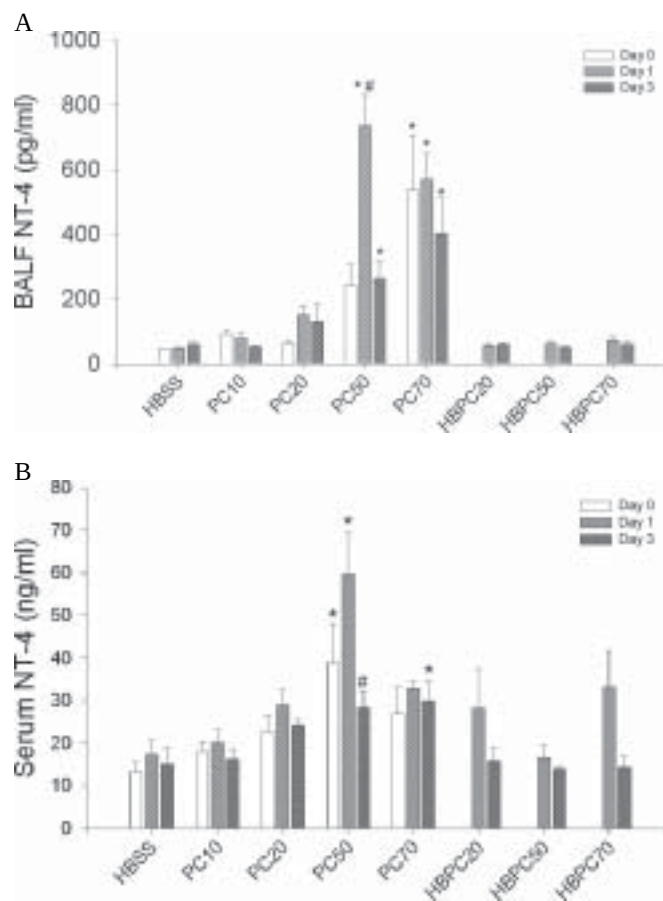


FIG. 2. NT-4 levels: (A) BALF NT-4, (B) serum NT-4. Mice were exposed 4 times to PCE and compared to naive (HBSS) mice at d 0, 1, and 3 after the final exposure. NT-4 levels were measured by ELISA. Asterisk indicates significant difference from HBSS control within time points ($p < .05$). (#) Significant difference from d 0 control within treatment group ($p < .05$). Error bars represent standard error of the mean; $n = 4-5$. PC 10, 10 μ g *P. chrysogenum* extract (PCE); PC 20, 20 μ g PCE; PC 50, 50 μ g PCE; PC 70, 70 μ g PCE.

BDNF Levels

No change in BDNF levels was found between PC-treated and control mice (data not shown).

Immunohistochemistry of NGF

Mononuclear cells, eosinophils, and macrophages were the major infiltrated immune cells in the lungs of mice seen after multiple exposures to PCE (Chung et al. 2005). Inflammatory and structural cells including macrophages, mast cells, eosinophils, T and B lymphocytes, epithelial cells, smooth muscle, and fibroblasts are known sources of neurotrophins in allergic airways disease (Forssard et al., 2004). A moderate NGF immunoreactivity was observed in ciliated bronchial epithelium of HBSS controls at D0 (Figure 4A). An intense NGF immunoreactivity in infiltrated immune cells including

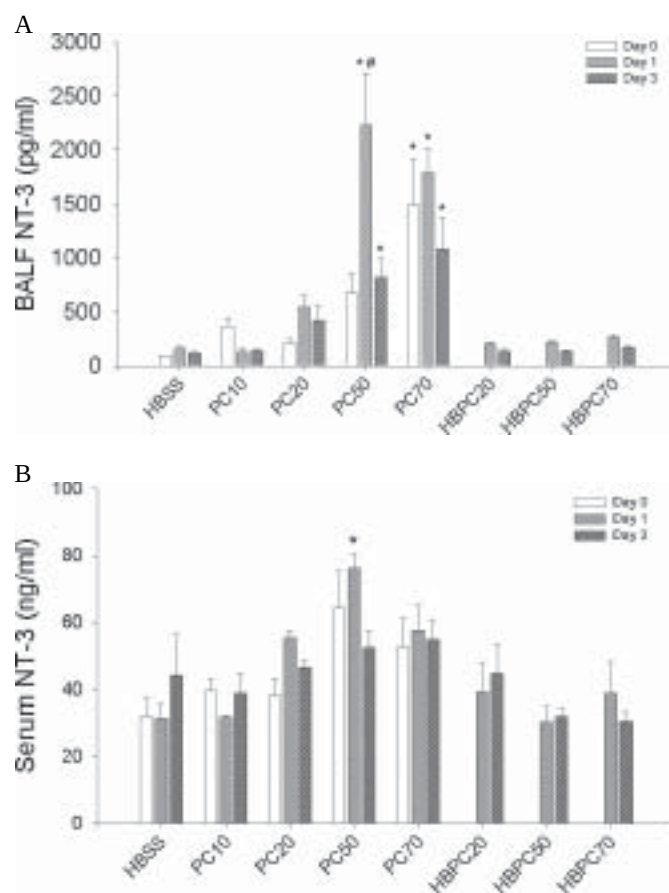


FIG. 3. NT-3 levels: (A) BALF NT-3, (B) serum NT-3. Mice were exposed 4 times to PCE and compared to naive (HBSS) mice at d 0, 1, and 3 after the final exposure. NT-3 levels were measured by ELISA. Asterisk indicates significant difference from HBSS control within time points ($p < .05$). #, Significant difference from d 0 control within treatment group ($p < .05$). Error bars represent standard error of the mean; $n = 4-5$. PC 10, 10 μ g *P. chrysogenum* extract (PCE); PC 20, 20 μ g PCE; PC 50, 50 μ g PCE; PC 70, 70 μ g PCE.

eosinophils and lymphocytes was noticeable in PC50-treated mice at D0 and D1 (Figure 4, B and C, respectively). There was no evidence of infiltrated immune cells in HBSS-treated mice.

Our previous study (Chung et al., 2005) showed that eosinophils, lymphocytes, and macrophages were dose-dependently increased in BALF with the maximum counts of the inflammatory cells at the PC50 dose. A correlation analysis between NTs in BALF and differential cell counts for d 1 indicated that eosinophils and lymphocytes were associated with the elevations of NGF with r^2 values of .81 and .85, NT-3 with .94 and .95, and NT-4 with .92 and .93, respectively.

Lungs of treated animals were lavaged and fixed for immunohistochemistry. In the lungs from HBSS-treated (control) animals in this study, epithelial cells stained positive for NGF, which indicates that this procedure did not alter the epithelial layer and was appropriate for our study. Although lavaging does in fact remove inflammatory cells from the airway lumen,

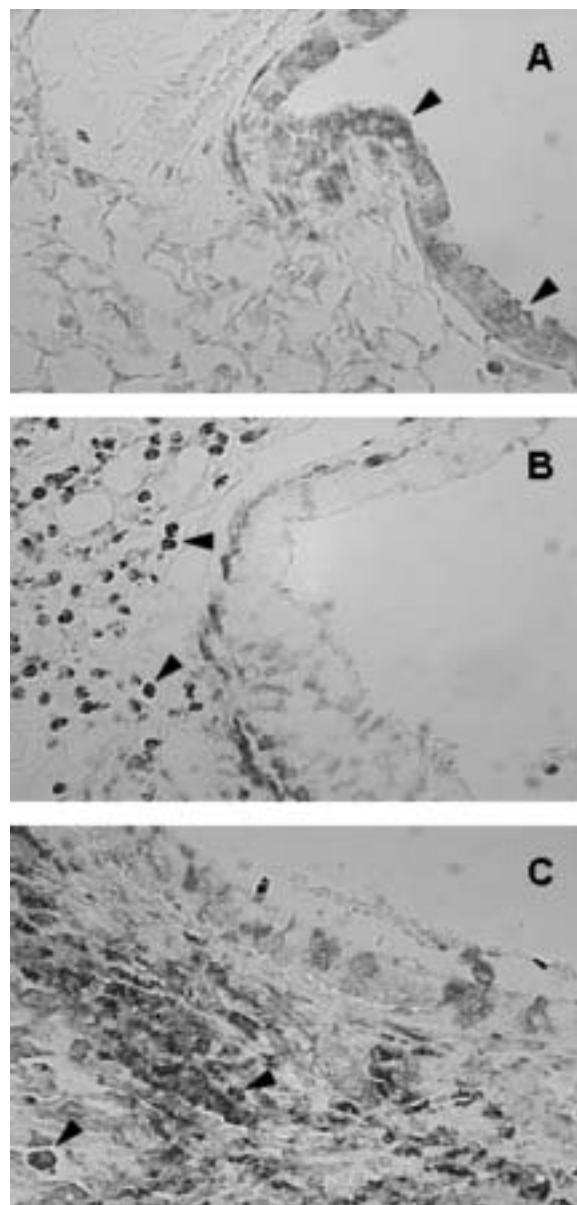


FIG. 4. Immunolocalization of NGF on lung sections with 40 $\times$ magnification from mice exposed 4 times to (A) HBSS at d 0 and (B) PC50 at d 0 and (C) d 1. Arrows indicate NGF positively staining cells. Moderate labeling was observed in bronchial epithelium (A) and an intense labeling in infiltrated inflammatory cells (B and C).

it does not have an effect on infiltrated cells in the interstitium. There was evidence for this in the interstitium of PCE-exposed lungs, where there was positive NGF staining in infiltrated inflammatory cells.

DISCUSSION

Neurotrophins have been linked to the etiology of asthma. In addition, NTs have been implicated in the exacerbation of asthma due to exposure to foreign agents such as respiratory

syncytial virus and diesel exhaust particulate matter (Piedimonte, 2003; Farraj et al., 2006). Mold exposure has also been linked to the causation and exacerbation of asthma. Recently, our laboratory found that multiple pulmonary challenges with *Penicillium chrysogenum* extract (PCE) induced allergic airways disease that is characteristic of asthma (Chung et al., 2005). The relationship between PCE-induced asthma and neurotrophins, however, is unknown. In the present study, data showed that the neurotrophins NGF, NT-3, and NT-4 were significantly elevated in the BALF and serum of mice treated with the highest doses of PCE (PC50, PC70). However, there was no effect of PCE on BDNF levels in both serum and BALF. Additionally, the infiltrating immune cells including mononuclear cells, eosinophils, and macrophages were found to be a source of NGF in this model.

Immunoglobulin E (IgE) is an important mediator of allergic airways symptoms. Binding of allergens to IgE activates mast cells or basophils, resulting in a systemic inflammatory response, which can produce life-threatening anaphylactic shock. Neurotrophins may influence IgE and/or mast cell activity. The mast cell expresses the trkA receptor and IgE binding induces mast cell release of NGF (Kassel et al., 2001; Xiang & Nilsson, 2000). Sera and BALF levels of IgE were dose-dependently increased in PCE-treated mice (Chung et al., 2005). In that study, the serum IgE levels were dose-dependently increased and significantly elevated in PC50 mice when compared to vehicle control mice. This rise in IgE paralleled the dose-dependent increase in NGF, NT-3, and NT-4 in the BALF. The parallel increases in IgE and NT levels are consistent with the findings in other mouse models of allergic asthma. Virchow et al. (1998) showed that the elevated NGF concentration in BALF following segmental allergen provocation of asthmatics was related to the total IgE level in sera of these asthmatics. Braun et al. (1998) showed that ovalbumin-challenged mice had increased levels of BALF NGF as well as total serum IgE. They suggested that the local rise in NGF levels in the lung was possibly due to the infiltration of immune cells and may stimulate B- and T-cell functions, resulting in a systemic increase in IgE levels. The upregulation in NT levels in this study may have mediated the PCE-induced increase in IgE.

A major source for elevated lung neurotrophin levels is infiltrating inflammatory cells such as eosinophils and lymphocytes (Kobayashi et al. 2002; Bonini et al., 1999). In the present study, mice treated with HBSS displayed a moderate staining for NGF in the bronchial epithelium and in airway macrophages. Mice treated with PCE, however, had a large number of infiltrated cells such as macrophages, lymphocytes, and eosinophils that were immunopositive for NGF. This finding suggests that the source of increased NGF in the BALF taken from PCE-treated mice may to a large extent derive from infiltrated inflammatory cells. These findings are in agreement with the results obtained by Olgart Hoglund et al. (2002), in which NGF-positive cells in the submucosa were increased in bronchial biopsy sections of asthmatics.

In conclusion, it was found that NTs are increased in a murine model of mold-induced allergic asthma and the potential cellular sources that may account for these increases were suggested. Additional research using antibodies against neurotrophins and/or their receptors and/or using neurotrophin knockout mice is required to further elucidate the functional roles of NTs in mold-induced allergic asthma.

REFERENCES

- Bonini, S., Lambiase, A., Levi-Schaffer, F., and Aloe, L. 1999. Nerve growth factor: An important molecule in allergic inflammation and tissue remodeling. *Int. Arch. Allergy Immunol.* 118:159–162.
- Braun, A., Appel, E., Baruch, R., Herz, U., Botchkarev, V., Paus, R., Brodie, C., and Renz, H. 1998. Role of nerve growth factor in a mouse model of allergic airway inflammation and asthma. *Eur. J. Immunol.* 28:3240–3251.
- Braun, A., Quarcoo, D., Schulte-Herbruggen, O., Lommatzsch, M., Hoyle, G., and Renz, H. 2001. Nerve growth factor induces airway hyperresponsiveness in mice. *Int. Arch. Allergy Immunol.* 124:205–207.
- Chou, H., Chang, C. Y., Tsai, J. J., Tang, R. B., Lee, S. S., Wang, S. R., Peng, H. J., and Shen, H. D. 2003. The prevalence of IgE antibody reactivity against the alkaline serine protease major allergen of *Penicillium chrysogenum* increases with the age of asthmatic patients. *Ann. Allergy Asthma Immunol.* 90:248–253.
- Chung, Y. J., Coates, N. H., Viana, M. E., Copeland, L., Vesper, S. J., Selgrade, M. K., and Ward, M. D. 2005. Dose-dependent allergic responses to an extract of *Penicillium chrysogenum* in BALB/c mice. *Toxicology* 216:73–84.
- Cooley, J. D., Wong, W. C., Jumper, C. A., Hutson, J. C., Williams, H. J., Schwab, C. J., and Straus, D. C. 2000. An animal model for allergic penicilliosis induced by the intranasal instillation of viable *Penicillium chrysogenum* conidia. *Thorax* 55:489–496.
- De Vries, A., Engels, F., Henricks, P. A., Leusink-Muis, T., Fischer, A., and Nijkamp, F. P. 2002. Antibodies directed against nerve growth factor inhibit the acute bronchoconstriction due to allergen challenge in guinea-pigs. *Clin. Exp. Allergy* 32:325–328.
- 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/B16J mice. *Inhal. Toxicol.* 18:483–491.
- Fox, A. J., Patel, H. J., Barnes, P. J., and Belvisi, M. G. 2001. Release of nerve growth factor by human pulmonary epithelial cells: role in airway inflammatory diseases. *Eur. J. Pharmacol.* 424:159–162.
- Freund, V., Pons, F., Joly, V., Mathieu, E., Martinet, N., and Frossard, N. 2002. Upregulation of nerve growth factor expression by human airway smooth muscle cells in inflammatory conditions. *Eur. Respir. J.* 20:458–463.
- Frossard, N., Freund, V., and Advenier, C. 2004. Nerve growth factor and its receptors in asthma and inflammation. *Eur. J. Pharmacol.* 500:453–465.
- Hudson, B., Flemming, J., Sun, G., and Rand, T. G. 2005. Comparison of immunomodulator mRNA and protein expression in the lungs of *Stachybotrys chartarum* spore-exposed mice. *J. Toxicol. Environ. Health A* 68:1321–1335.
- 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.
- Kobayashi, H., Gleich, G. J., Butterfield, J. H., and Kita, H. 2002. Human eosinophils produce neurotrophins and secrete nerve growth factor on immunologic stimuli. *Blood* 9:2214–2220.
- Lewin, G. R., and Barde, Y. A. 1996. Physiology of the neurotrophins. *Annu. Rev. Neurosci.* 19:289–317.
- 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.
- Nassenstein, C., Kerzel, S., and Braun, A. 2004. Neurotrophins and neurotrophin receptors in allergic asthma. *Prog. Brain Res.* 146:347–367.
- Noga, O., Englmann, C., Hanf, G., Grutzkau, A., Guhl, S., and Kunkel, G. 2002. Activation of the specific neurotrophin receptors TrkA, TrkB and TrkC influences the function of eosinophils. *Clin. Exp. Allergy* 32:1348–1354.
- Noga, O., Englmann, C., Hanf, G., Grutzkau, A., Seybold, J., and Kunkel, G. 2003. The production, storage and release of the neurotrophins nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 by human peripheral eosinophils in allergics and non-allergics. *Clin. Exp. Allergy* 33:649–654.
- Olgart, C., and Frossard, N. 2001. Human lung fibroblasts secrete nerve growth factor: effect of inflammatory cytokines and glucocorticoids. *Eur. Respir. J.* 18:115–121.
- 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.
- 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.
- Piedimonte, G. 2003. Contribution of neuroimmune mechanisms to airway inflammation and remodeling during and after respiratory syncytial virus infection. *Pediatr. Infect. Dis. J.* 22:S66–S74; discussion S74–S75.
- Rand, T. G., Flemming, J., Miller, J. D., and Womiloju, T. O. 2006. Comparison of inflammatory responses in mouse lungs exposed to *Atraneos* A and C from *Stachybotrys chartarum*. *J. Toxicol. Environ. Health A* 69:1239–1251.
- Rivera, A., Van Epps, H. L., Hohl, T. M., Rizzuto, G., and Pamer, E. G. 2005. Distinct CD4⁺-T-cell responses to live and heat-inactivated *Aspergillus fumigatus* conidia. *Infect. Immun.* 73:7170–7179.
- Schelenz, S., Smith, D. A., and Bancroft, G. J. 1999. Cytokine and chemokine responses following pulmonary challenge with *Aspergillus fumigatus*: Obligatory role of TNF- α and GM-CSF in neutrophil recruitment. *Med. Mycol.* 37:183–194.
- Schwab, C. J., Cooley, J. D., Brasel, T., Jumper, C. A., Graham, S. C., and Straus, D. C. 2003. Characterization of exposure to low levels of viable *Penicillium chrysogenum* conidia and allergic sensitization induced by a protease allergen extract from viable *P. chrysogenum* conidia in mice. *Int. Arch. Allergy Immunol.* 130:200–208.
- 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.
- Ward, M. D., Madison, S. L., Sailstad, D. M., Gavett, S. H., and Selgrade, M. K. 2000. Allergen-triggered airway hyperresponsiveness and lung pathology in mice sensitized with the biopesticide *Metarhizium anisopliae*. *Toxicology* 143:141–154.
- Ward, M. D., Sailstad, D. M., and Selgrade, M. K. 1998. Allergic responses to the biopesticide *Metarhizium anisopliae* in Balb/c mice. *Toxicol. Sci.* 45:195–203.
- Xiang, Z., and Nilsson, G. 2000. IgE receptor-mediated release of nerve growth factor by mast cells. *Clin. Exp. Allergy* 30:1379–1386.