

Neurotrophins Are Increased in Bronchoalveolar Lavage Fluid after Segmental Allergen Provocation

JOHANN CHRISTIAN VIRCHOW, PETER JULIUS, MAREK LOMMATZSCH, WERNER LUTTMANN, HARALD RENZ, and ARMIN BRAUN

Institute of Laboratory Medicine and Pathobiochemistry, Charité-Campus Virchow-Klinikum, Humboldt University, Berlin; and Department of Pneumology, University Medical Clinic, Albert-Ludwigs University, Freiburg, Germany

The mechanisms linking inflammation and airway hyperresponsiveness in allergic bronchial asthma are still not completely defined. Since neurotrophic factors increase nerve excitability and neurotransmitter synthesis and are produced by immunocompetent cells, they are likely candidates as mediators of inflammation and hyperresponsiveness. We tested the hypothesis that neurotrophin concentrations will increase in the bronchoalveolar lavage (BAL) fluid from patients with asthma after segmental allergen provocation. For this purpose an individually standardized dose of allergen or saline was instilled into different segments during bronchoscopy in eight subjects with mild allergic bronchial asthma. Segments were then lavaged 10 min and 18 h after allergen challenge or saline instillation. There was a significant increase in the neurotrophins nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 in BAL fluids 18 h after allergen but not saline challenge. We conclude that neurotrophins are produced endobronchially following allergen provocation, suggesting a contribution to the pathogenesis of asthma. Virchow JC, Julius P, Lommatzsch M, Luttmann W, Renz H, Braun A. Neurotrophins are increased in bronchoalveolar lavage fluid after segmental allergen provocation.

AM J RESPIR CRIT CARE MED 1998;158:2002-2005.

Airway smooth muscle tone is controlled by sympathetic and parasympathetic nerves as well as by peptidergic nerves of the nonadrenergic, noncholinergic (NANC) system. It has been suggested that neuropeptides released from NANC neurons could contribute to the inflammatory response in asthma by axon reflexes (1). Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) are neurotrophins belonging to a family of mediators that exert their effects in both the nervous system and the immune system. The functions of the neurotrophins in survival, development, differentiation, and maintenance of nerve cells are well defined (2). In addition to their effects in the central nervous system (CNS), neurotrophins also affect peripheral sympathetic and peptidergic neurons. Neurotrophins upregulate neuropeptide production in sensory neurons and contribute to inflammatory hypersensitivity (3). Neurotrophin receptors are expressed on nerve cells as well as on immune cells, including monocytes, mast cells, B cells, and T cells. Several immunologic functions have been described for NGF, such as induction of mast cell degranulation, induction of cytokine synthesis, and regulation of antibody production (4). Additionally, NGF is a chemoattractant and activation factor for eosinophils (5). Under physiologic conditions, neurotrophins are

produced by nerve-associated cells like glia cells or Schwann cells and by nerve cells themselves (6), while during inflammation neurotrophins can be also produced by fibroblasts (7), mast cells, macrophages, and T and B cells (8). In a recent study high serum levels of NGF were detected in patients with severe allergic bronchial asthma (9). Our aim was to evaluate the local production of neurotrophins at the site of allergic inflammation in bronchial asthma. For this purpose, a well-defined and standardized protocol of segmental allergen challenge in patients with asthma was used (10).

METHODS

Subjects

We studied eight nonsmoking, mildly allergic male subjects with asthma with a mean age of 24.3 ± 2.4 yr (range, 21-29 yr) and a mean FEV₁ of $97.3 \pm 9.6\%$ (82-113%) of predicted (11). Mean total immunoglobulin E (IgE) was 583.7 ± 598.8 kilo units per liter (kU/L), and all patients had positive reactions to the skin prick test and elevated specific IgE (31.0 ± 29.5 kU/L) to at least one common aeroallergen. All subjects had a history of intermittent wheeze, chest tightness, cough, and sputum production and reversible bronchoconstriction after inhalation of allergens. There was no evidence suggesting a respiratory tract infection before or at the time of the segmental allergen challenge. All subjects received inhaled β_2 -agonist therapy as needed. Cromoglycate (n = 3) and inhaled corticosteroids (n = 1) were withdrawn 7 d prior to the study. All subjects gave informed consent. The study protocol was approved by the Ethics Committee of the University of Freiburg.

Inhaled Allergen Provocation

The inhaled allergen provocation was performed as described before (12). The individual provocative concentration of methacholine that caused a 20% fall in FEV₁ (PC₂₀ micro Biological Units [mBU] of al-

(Received in original form March 5, 1998 and in revised form July 30, 1998)

Supported by the Volkswagen Stiftung and by a grant from the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF 01 GC 9701/7).

Correspondence and requests for reprints should be addressed to Harald Renz, M.D., Charité-Campus Virchow-Klinikum, Department of Laboratory Medicine and Pathobiochemistry, Augustenburger Platz 1, 13353 Berlin, Germany. E-mail: abraun@ukrv.de

Am J Respir Crit Care Med Vol 158, pp 2002-2005, 1998
Internet address: www.atsjournals.org

lergen) was extrapolated for each patient according to the cumulative dose of allergen inhaled until a drop in FEV₁ of more than 20% was recorded. A 10-fold higher dose of allergen was then used for the subsequent segmental allergen provocation. Inhaled and segmental allergen provocations were at least 3 wk apart.

Segmental Allergen Challenge

Bronchoscopy and segmental allergen challenge were performed as previously described (10, 12). As a control, 2.5 ml of saline were instilled into the inferior lingular bronchus (B5 left). Furthermore, in six subjects 2.5 ml of saline were instilled into one segment of the lower

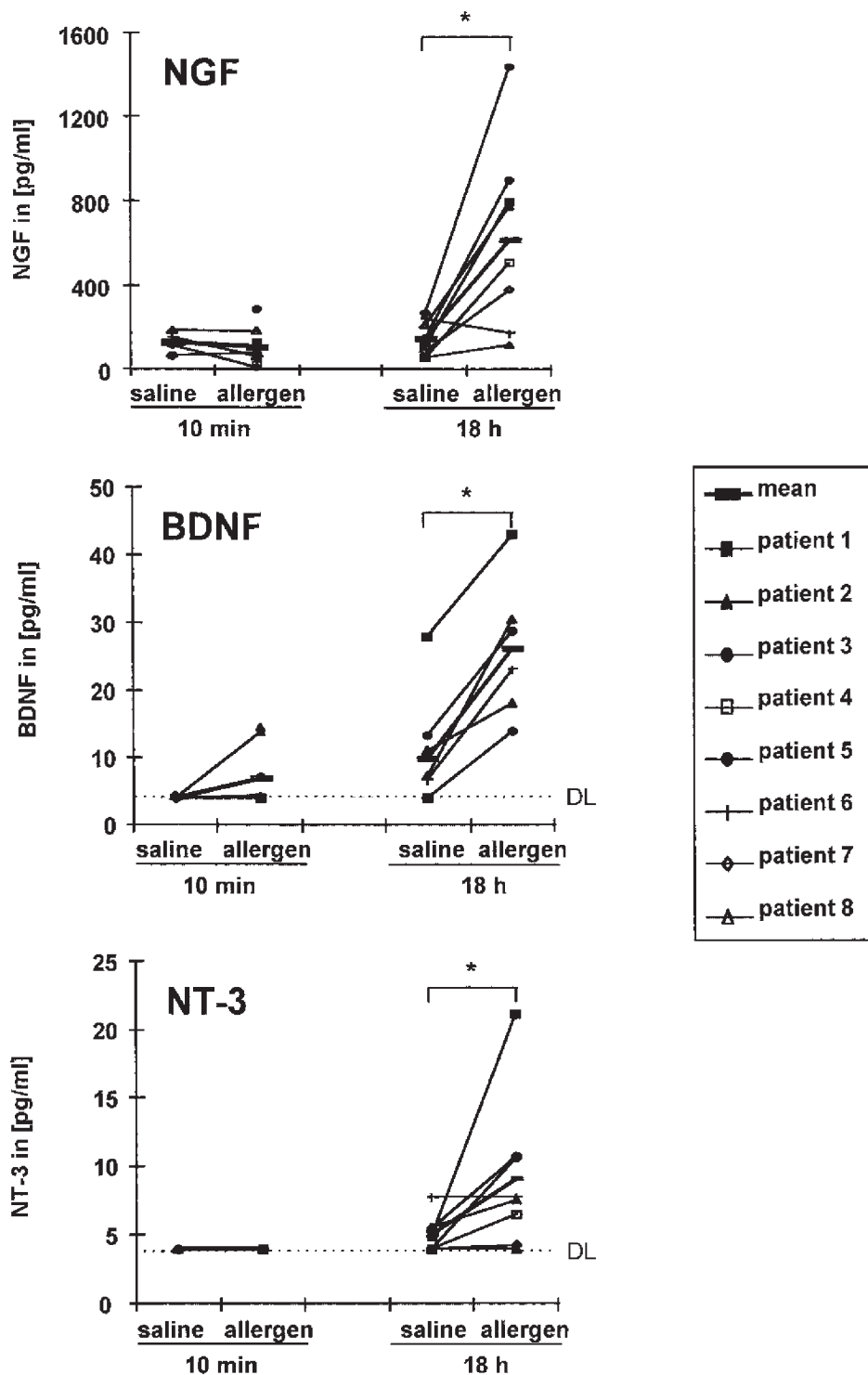


Figure 1. Neurotrophin content in BAL fluid 18 h after segmental provocation. Saline-challenged segment lavaged 10 min or 18 h after instillation of 2.5 ml normal saline was compared with allergen-challenged segment lavaged 10 min or 18 h after instillation of allergen 10 × PD₂₀ or 50 protein nitrogen units (PNU), respectively, for each patient. *Significant differences (p < 0.05) between groups. Means are given in bold lines.

left lobe, which was lavaged after 10 min. Lavage was performed using 100 ml of normal, prewarmed saline. Allergen (rye pollen, birch pollen, or house dust mite allergen) was diluted in 2.5 ml of saline and instilled into segments (B7 and B5 right). The right lower lobe bronchus was lavaged 10 min after endoscopic allergen deposition. After 18 h bronchoscopy was performed again, and the left lingular bronchus in which 2.5 ml diluent had been instilled earlier was lavaged; subsequently the medial segment of the right middle lobe bronchus was identified and lavaged using the same technique as described previously.

Analysis of Bronchoalveolar Lavage Leukocytes

Bronchoalveolar lavage (BAL) samples were filtered through a two-layer sterile gauze into sterile plastic vials (Falcon, Oxnard, CA), centrifuged at 4°C and 500 × g for 10 min. The supernatant was removed and stored at -70°C. Differential cell counts were performed on all nucleated cells and results expressed as total number of cells per microliter of recovered fluid.

Determination of NGF, BDNF, and NT-3 by ELISA

NGF, BDNF, and NT-3 were measured in cell-free BAL fluids with commercial ELISA kits according to the manufacturer's instructions (Promega, Madison, WI). Measurements were performed in duplicates and are expressed as means.

Statistical Analysis

Results are expressed as arithmetic mean with standard deviation. Differences between groups were analyzed using the Wilcoxon matched pairs test. Differences with p values < 0.05 were considered statistically significant.

RESULTS

Neurotrophin Content in BAL

As shown previously (12), segmental allergen challenge triggers airway inflammation as indicated by a significant increase in eosinophils (p < 0.05) and neutrophils (p < 0.05) at 18 h compared with the 10-min postallergen challenge. NGF was detected in all BAL samples. There was no significant difference between NGF concentrations measured in BAL fluids 10 min after saline or allergen challenge (Figure 1). In contrast, at 18 h there was an approximately fourfold increase in the NGF concentrations following allergen provocation, but not in the saline group (p < 0.05). Highest levels of BDNF were measured 18 h after allergen challenge. Although BDNF was also increased in the saline group, the concentrations were significantly lower (p < 0.05). NT-3 was not present in BAL samples at 10 min, regardless of whether they were obtained after saline or allergen challenge. Only in the segments that were lavaged 18 h after allergen provocation could elevated concentrations of NT-3 be detected (Figure 1).

DISCUSSION

We investigated the hypothesis that neurotrophins are produced locally following allergen challenge in allergic asthma. We found a marked upregulation of neurotrophins 18 h after allergen exposure but not after saline challenge. This finding coincides with the demonstration of activated T cells and eosinophils in BAL fluid following allergen challenge (10). Since the concentrations of neurotrophins increased markedly in the allergen-challenged segments but remained basically unchanged in the saline-challenged segments, we conclude that enhanced neurotrophin production occurs in a selective and allergen-dependent fashion during the allergic late-phase response, and our data support the hypothesis that these mediators are produced locally during allergic inflammation.

Although NGF can be released from mast cells, macrophages, T cells and B cells (8) as well as fibroblasts (7) and epithelial cells, the source of neurotrophins in this model of seg-

mental allergen challenge remains to be determined. NGF and BDNF have been shown to increase sensitivity and excitability of sensory and motor neurons (13). In addition, neurotrophins are potent inducers of neuropeptide expression (e.g., substance P) in sensory neurons (3) and contribute to inflammatory sensory hypersensitivity. These neuropeptides, which belong to the tachykinin family and are elevated in BAL of patients with asthma (14), mediate important biologic activities during acute inflammatory responses, including smooth muscle contraction, dilatation, and increased permeability of blood vessels (1), all of which contribute to the severity of asthmatic bronchoconstriction.

Studies in mice support our hypothesis that neurotrophins are causally related to bronchial hyperreactivity. Transgenic mice overexpressing NGF in the lung will develop hyperinnervation of the airways, enhanced neuropeptide levels in the lung, and airway hyperreactivity (15). To further analyze the potential role of NGF in allergy, we have treated mice that developed allergen-induced airway inflammation and airway hyperresponsiveness with anti-NGF. Local intranasal anti-NGF completely prevented development of airway hyperresponsiveness, whereas there was little effect on airway inflammation (16). Yet the relative contribution of NGF to human bronchial asthma remains to be investigated.

In conclusion, we have demonstrated local upregulation of neurotrophin production in allergic inflammation after allergen provocation. This increase of neurotrophin production occurred following the late-phase response. Our data therefore suggest that allergic asthma is associated with a dysregulation of the complex interactions between the nervous and immune system. Functional studies are currently designed to analyze the relevance of this phenomenon for human bronchial asthma.

References

- Barnes, P. J. 1996. Neuroeffector mechanisms: the interface between inflammation and neuronal responses. *J. Allergy Clin. Immunol.* 98:S73-S81.
- Lewin, G. R., and Y. A. Barde. 1996. Physiology of the neurotrophins. *Annu. Rev. Neurosci.* 19:289-317.
- Donnerer, J., R. Schuligoi, and C. Stein. 1992. Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in vivo. *Neuroscience* 49:693-698.
- Aloe, L., L. Bracci Laudiero, S. Bonini, and L. Manni. 1997. The expanding role of nerve growth factor: from neurotrophic activity to immunologic diseases. *Allergy* 52:883-894.
- Hamada, A., N. Watanabe, H. Ohtomo, and H. Matsuda. 1996. Nerve growth factor enhances survival and cytotoxic activity of human eosinophils. *Br. J. Haematol.* 93:299-302.
- Meyer, M., I. Matsuoka, C. Wetmore, L. Olson, and H. Thoenen. 1992. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J. Cell Biol.* 119:45-54.
- Lindholm, D., R. Heumann, M. Meyer, and H. Thoenen. 1987. Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature* 330:658-659.
- Otten, U., J. L. Scully, P. B. Ehrhard, and R. A. Gadiant. 1994. Neurotrophins: signals between the nervous and immune systems. *Prog. Brain Res.* 103:293-305.
- Bonini, S., A. Lambiase, S. Bonini, F. Angelucci, L. Margrini, L. Manni, and L. Aloe. 1996. Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc. Natl. Acad. Sci. U.S.A.* 93:10955-10960.
- Virchow, J. C., Jr., C. Walker, D. Hafner, C. Kortsik, P. Werner, H. Mathys, and C. Kroegel. 1995. T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. *Am. J. Respir. Crit. Care Med.* 151:960-968.
- National Institutes of Health. 1992. International Consensus Report on Diagnosis and Treatment of Asthma. National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD. Publica-

- tion No. 92-3091.
12. Virchow, J. C., P. Julius, H. Matthys, C. Kroegel, and W. Luttmann. 1998. CD14 expression and soluble CD14 after segmental allergen provocation in atopic asthma. *Eur. Respir. J.* 11:317-323.
 13. Gonzalez, M., and W. F. Collins. 1997. Modulation of motoneuron excitability by brain-derived neurotrophic factor. *J. Neurophysiol.* 77:502-506.
 14. Nieber, K., C. R. Baumgarten, R. Rathsack, J. Furkert, P. Oehme, and G. Kunkel. 1992. Substance P and beta-endorphin-like immunoreactivity in lavage fluids of subjects with and without allergic asthma. *J. Allergy Clin. Immunol.* 90:646-652.
 15. Hoyle, G. W., R. M. Graham, J. B. Finkelstein, K. P. T. Nguyen, D. Gozal, and M. Friedman. 1998. Hyperinnervation of the airways in transgenic mice overexpressing nerve growth factor. *Am. J. Respir. Cell Mol. Biol.* 18:149-157.
 16. Braun, A., E. Appel, R. Baruch, U. Herz, C. Brodie, and H. Renz. 1998. The role of nerve growth factor in a mouse model of allergic airway inflammation and asthma. *Eur. J. Immunol.* (In press)