Eur Respir J 2005; 26: 15–20
DOI: 10.1183/09031936.05.00047804

Nerve growth factor is released by IL-1β and induces hyperresponsiveness of the human isolated bronchus

N. Frossard*, E. Naline#, C. Olgart Höglund*, O. Georges¶ and C. Advenier#

ABSTRACT: Nerve growth factor (NGF) is a neurotrophic factor essential for the development and survival of neurons, and is also an important mediator of inflammation. It is released by airway cells stimulated by interleukin (IL)-1β. As IL-1β induces airway hyperresponsiveness (AHR) to the tachykinin NK-1 receptor agonist [Sar^9,$Met(O_2)^{11}$]-substance P in human isolated bronchi, the aim of this study was to determine whether IL-1β was able to induce NGF release from isolated bronchi, and whether NGF might participate into IL-1β-induced AHR.

IL-1β (10 ng·mL^{-1}; 21°C; 15 h) increased the release of NGF from human isolated bronchi *in vitro*, and, in organ bath studies, the response of human bronchi to [Sar^9,$Met(O_2)^{11}$]-substance P (0.1 μm). A significant correlation was found between these responses. AHR induced by IL-1β was abolished by a blocking anti-human NGF antibody. Finally, NGF (1 ng·mL^{-1}; 37°C; 0.5 h) by itself induced a significant increase in [Sar^9,$Met(O_2)^{11}$]-substance P responsiveness. By contrast, it did not change the maximal contraction to acetylcholine.

In conclusion, the present study clearly demonstrated that nerve growth factor may participate in the airway hyperresponsiveness induced by interleukin-1β, which supports the neuro-immune cross-talk that may be active in the development of hyperresponsiveness in the human airways, and suggests nerve growth factor is active in the airways in asthma.

KEYWORDS: Asthma, bronchial hyperresponsiveness, inflammation, nerve growth factor, neurotrophin

Nerve growth factor (NGF) is a neurotrophic factor essential for the development and survival of neurons [1, 2]. It has recently been suggested to function as an important mediator of inflammation [3–5]. In the airways in particular, animal studies have shown NGF to contribute to the development of airway hyperresponsiveness (AHR). First, NGF blocking antibodies abolish the AHR created in sensitised and challenged mice [6]. In addition, AHR is induced by tissue-specific overexpression of NGF in the airways [7, 8]. Also, NGF by itself induces hyperresponsiveness of the guinea pig airways *in vitro* [9] and *in vivo* [10]. However, the evidence of NGF contributing to AHR in humans is lacking, although expression of NGF is increased in asthma [4, 5].

Previous studies reported that interleukin (IL)-1β induces AHR in several animal models [11–13]. In addition, BARCHASZ *et al.* [14] described that IL-1β causes AHR to the NK-1 tachykinin receptor agonist [Sar^9,$Met(O_2)^{11}$]-substance P (SP) in the human isolated bronchus. The mechanism of such hyperresponsiveness is unclear. In experiments performed in human airway cells in culture, IL-1β induced the release of NGF from fibroblasts [15], bronchial smooth muscle cells [16] and airway epithelial cells [17, 18]. However, whether IL-1β is able to induce release of NGF from the human bronchus *ex vivo* has not yet been reported.

Therefore, the aim of the present study was to determine whether: 1) IL-1β is able to induce release of NGF from the human isolated bronchus, and 2) NGF participates in AHR.

METHODS

Preparation of human bronchial tissue

Bronchial tissues were surgically removed from 42 patients with lung cancer (27 males, 15 females; mean±SD age 61.0±1.8 yrs). The protocol was approved by a local ethics committee (Comité de Protection des Personnes se Prêtant à la Recherche Biomédicale, Versailles, France). After resection, segments of human bronchi were taken and placed in oxygenated Krebs-Henseleit solution (composition: NaCl 119 mM, KCl

AFFILIATIONS
*EA 3771, Inflammation and environment in asthma, Université Louis Pasteur, Strasbourg, and #UPRES EA220, Université de Versailles and UFR Biomédicale des Saints-Pères, and ¶Laboratoire d'anatomo-pathologie Guigui-Georges, Paris, France.

CORRESPONDENCE
N. Frossard
EA 3771
Inflammation and environment in asthma
Faculté de pharmacie
Université Louis Pasteur
BP 60024
67401 Illkirch Cedex
France
Fax: 33 390244309
E-mail: nelly.frossard@pharma.u-strasbg.fr

Received:
April 23 2004
Accepted after revision:
March 16 2005

SUPPORT STATEMENT
C. Olgart Höglund was supported in this study by the Swedish Research Council (Stockholm, Sweden), Torsten and Ragnar Söderberg's Foundations (Sweden), Erik and Edith Fernström's Foundation (Sweden), Comité Départmental du Bas-Rhin Contre les Maladies Respiratories et la Tuberculose (France), and the UCB Institute of Allergy (Belgium). This study received support funding from Inserm (Illkirch Cedex, France) and Université Louis Pasteur (Strasbourg, France).

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

4.7 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM and glucose 11.7 mM). After removal of adhering lung parenchyma and connective tissue, rings of the same bronchus were prepared (5–7 mm length × 0.5–1 mm internal diameter), divided into paired groups and randomised [19]. The diameter was measured with a stage micrometer at light transmission after haematoxylin, eosin and saffron staining of 5 μm paraffin sections of a sample of bronchi fixed for 24 h in 10% formyl saline.

Bronchial ring segments were placed in 1 mL Krebs-Henseleit solution at room temperature (21℃) for 15 h in the presence or absence of IL-1β (10 ng·mL^{-1}) as previously reported [14, 15]. After incubation, the paired bronchi were taken for contractile studies, and the supernatants kept aliquoted at -80℃ until NGF measurement. In another set of experiments, anti-human NGF blocking antibody or an irrelevant immunoglobulin (Ig)-G antibody (200–500 ng·mL^{-1}; R&D Systems Europe, Lille, France) were incubated simultaneously to IL-1β for 15 h at 21℃, before bronchi were taken for contractile studies.

The effect of exogenous NGF (0.01–1 ng·mL^{-1}) was studied on bronchial ring segments kept in Krebs-Henseleit solution at 4℃ overnight. Contractile studies were performed after NGF pre-treatment for 30 min directly in the organ baths.

Experimental procedure

Bronchial ring segments were suspended in 5 mL organ baths containing Krebs-Henseleit solution, gassed with 95% O_2–5% CO_2 and maintained at 37℃. Each preparation was connected to an isometric force displacement transducer (UF1; Piodem, Canterbury, Kent, UK) and EMKA amplifier (EMKA Technology, Les Ulis, France). Changes in tension were recorded on a polygraph. Preparations were suspended with an initial tension of 1.5 g, washed three times every 10 min, and equilibrated for another 30 min at 1–1.5 g.

The contractile response to the tachykinin NK-1 receptor agonist, [Sar^9,Met(O$_2$)^{11}]-SP was studied. Bronchial ring segments were washed out and acetylcholine was applied for maximal contraction. A 0.1 μM concentration of [Sar^9,Met(O$_2$)^{11}]-SP was selected for maximal NK-1 contractile response without interference with the tachykinin NK-2 receptor, and a 3 mM acetylcholine concentration for maximal response [20].

Quantification of NGF protein by ELISA

NGF was quantified in the supernatant of human isolated bronchi pre-treated for 15 h with IL-1β (10 ng·mL^{-1}) or solvent. A commercially available NGF-specific, highly sensitive, two-site ELISA kit was used following the manufacturer's instructions (Promega, Madison, WI, USA). Briefly, 96-well immunoplates (Maxisorp™, Nunc, Roskilde, Denmark) were coated with a polyclonal goat anti-human NGF antibody in a coating buffer (25 mM carbonate buffer; pH 9.7). After overnight incubation at 4℃, plates were washed (20 mM Tris-HCl, 150 mM NaCl with 0.05% (v/v) Tween®-20; Sigma-Aldrich, St Louis, MO, USA), and incubated in a blocking buffer for 1 h. The supernatants and the standard recombinant human NGF dilutions were incubated at 37℃ for 6 h and washed. Rat monoclonal anti-human NGF antibody (0.25 μg·mL^{-1}) was added for overnight incubation at 4℃, and washed. Anti-rat horseradish peroxidase-conjugated IgG antibodies were incubated for 2.5 h, and the substrate (3,3′,5,5′-tetramethylbenzidine 0.02% and hydrogen peroxidase 0.01%) was added. The colorimetric reaction was stopped after 10 min by adding phosphoric acid (1 M), and the optical density was measured in duplicate at 450 nm. The detection range was 3.9–500 pg·mL^{-1}.

Expression of results and statistical analysis

All values are expressed as mean±SEM. Contractile responses were expressed in g and as a percentage of the maximal contraction induced by acetylcholine (3 mM). NGF protein levels were expressed as pg of NGF·mg^{-1} wet weight tissue. Differences between groups were analysed from raw data using an unpaired, two-tailed t-test, and a two-way ANOVA with Student-Newman-Keuls test when more than two variables were compared. Data were considered significantly different at $p<0.05$. The correlation between the NGF release (% increase) and contraction to [Sar^9,Met(O$_2$)^{11}]-SP (% increase) was evaluated using regression analysis. The coefficient of determination (r^2) was determined from the regression curve.

Drugs

The drugs used were: [Sar^9,Met(O$_2$)^{11}]-SP, recombinant human IL-1β (Bachem, Bubendorf, Switzerland), recombinant NGF, anti-human NGF blocking antibody and an irrelevant IgG antibody (R&D Systems Europe). IL-1β and NGF were dissolved in distilled water at a concentration of 0.075 μM and 20 μg·mL^{-1}, respectively, and kept aliquoted at -80℃ until use. All drugs were dissolved in distilled water and further diluted in Krebs-Henseleit solution.

RESULTS

Effect of IL-1β on NGF release and on AHR

As previously reported, the human isolated bronchus incubated with IL-1β became hyperresponsive to the NK-1 receptor agonist [Sar^9,Met(O$_2$)^{11}]-SP (0.1 μM). Response was increased by 64.8±13.3% ($p<0.001$; n=24; fig. 1). In contrast, the response to acetylcholine (3 mM) was unchanged (2.12±0.18 and 2.21±0.17 g for IL-1β and saline, respectively; n=24).

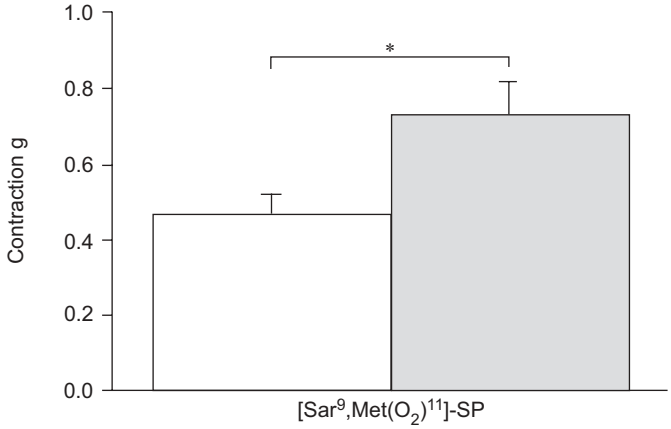

FIGURE 1. Effect of interleukin (IL)-1β (10 ng·mL^{-1}; 15 h; 21℃) on contractile responses induced by [Sar^9,Met(O$_2$)^{11}]-substance P (SP) (0.1 μM) on the human isolated bronchus. □: without IL-1β; ■: with IL-1β. n=24. *: $p<0.05$.

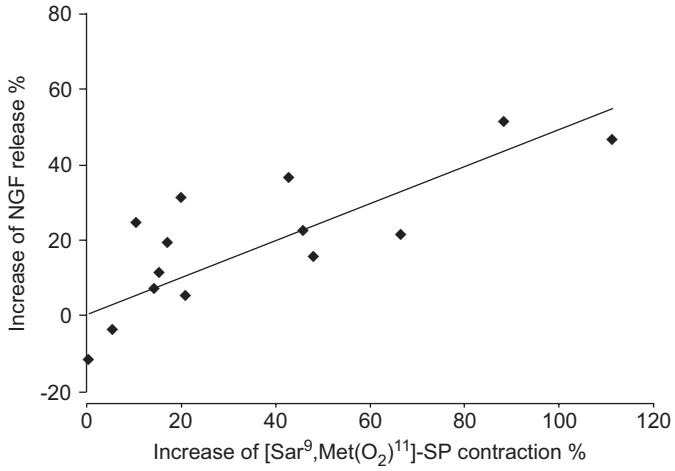

FIGURE 2. Correlation between the percentage of increase in contraction to [Sar^9,$Met(O_2)^{11}$]-substance P (SP) (0.1 μM) and the percentage increase in nerve growth factor (NGF) release induced by interleukin (IL)-1β.

In parallel, a significant increase in the levels of NGF, although slight, was measured in the incubation medium after treatment with IL-1β (3.1±0.5 and 2.5±0.5 pg·mg^{-1} wet weight tissue after IL-1β and saline, respectively; n=14; $p<0.05$), *i.e.* a 28.8±5.2% increase in NGF release. A positive correlation between NGF release and bronchial hyperresponsiveness to IL-1β was measured ($r^2=0.59$; $p<0.05$; fig. 2).

Effect of a NGF blocking antibody on AHR induced by IL-1β

Pre-incubation of an anti-human NGF blocking antibody (500 ng·mL^{-1}) simultaneously to IL-1β totally abolished the IL-1β-induced AHR to [Sar^9,$Met(O_2)^{11}$]-SP ($p<0.05$ for 50 ng·mL^{-1}; fig. 3), but had no effects on the contractile response to this NK-1 receptor agonist *per se*. Under similar conditions, pre-incubation with an irrelevant IgG antibody had no effect on the IL-1β-induced hyperresponsiveness to [Sar^9, $Met(O_2)^{11}$]-SP responses, or on the contractile response to this agonist.

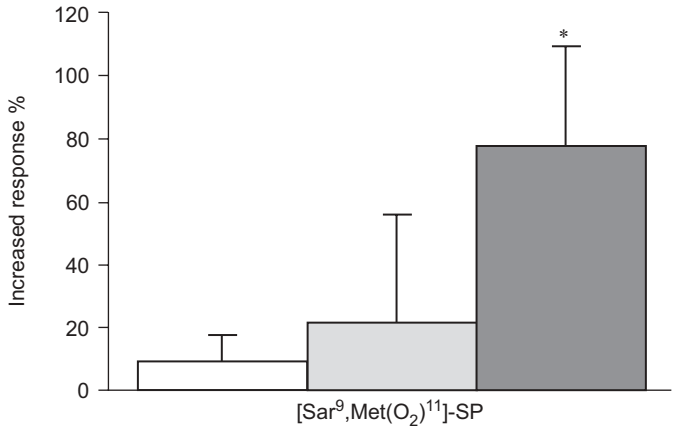

FIGURE 4. Effect of nerve growth factor (NGF; 0.01, 0.1, 1 ng·mL^{-1}; 30 min) on responses of human isolated bronchi to the tachykinin NK-1 receptor agonist [Sar^9,$Met(O_2)^{11}$]-substance P (SP) (0.1 μM). □: + NGF 0.01 ng·mL^{-1}; ▩: + NGF 0.1 ng·mL^{-1}; ■: + NGF 1 ng·mL^{-1}. Results are expressed as percentages of increase from control values (mean±SEM of n=11 experiments). *: $p<0.05$ *versus* control.

AHR to NGF

Exogenous NGF alone had no effect on bronchial smooth muscle tone. NGF (0.01–1 ng·ml^{-1}) induced a concentration-dependent increase in [Sar^9,$Met(O_2)^{11}$]-SP response, with a maximal increase of 77.0±31.5% at 1 ng·mL^{-1} ($p<0.05$; n=11; fig. 4). In contrast, NGF (1 ng·ml^{-1}) did not change the acetylcholine (3 mM)-induced contraction (2.7±6.1%, non-significant).

DISCUSSION

The present results show that IL-1β induces release of NGF from the human isolated bronchus, and this release is correlated with the increased response to [Sar^9,$Met(O_2)^{11}$]-SP induced by IL-1β. In addition, IL-1β-induced hyperresponsiveness is abolished by pre-treatment with an anti-NGF blocking antibody. Finally, NGF by itself induces

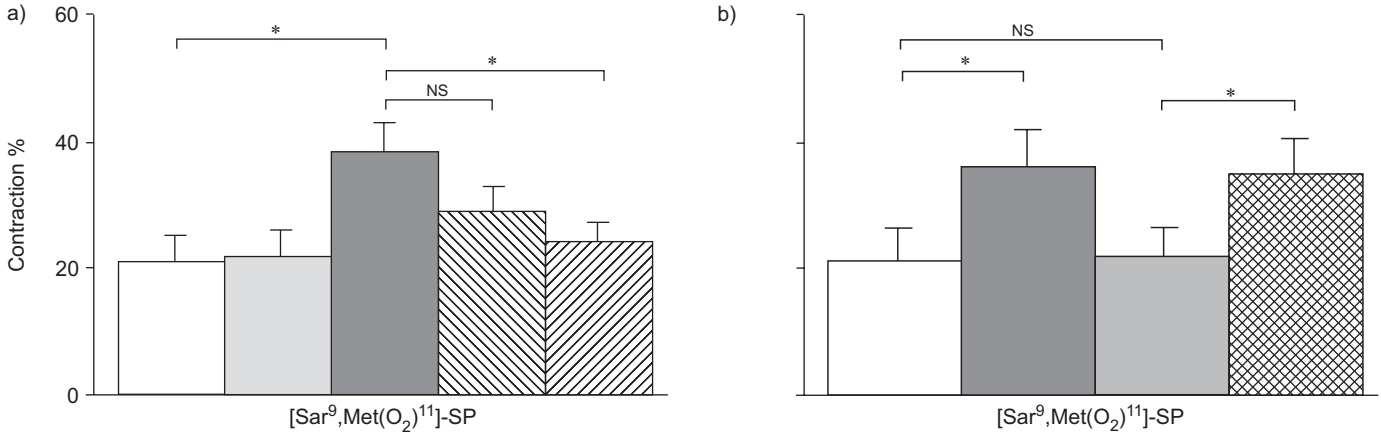

FIGURE 3. a) Effect of an anti-human NGF blocking antibody (anti-NGF; 200–500 ng·mL^{-1}). □: control; ▩: anti-NGF (500 ng·mL^{-1}); ■: interleukin (IL)-1β; ▧: IL-1β + anti-NGF (200 ng·mL^{-1}); ▨: IL-1β + anti-NGF (500 ng·mL^{-1}). b) Effect of an irrelevant immunoglobulin(Ig)-G (500 ng·mL^{-1}, 15 h, 21℃) on IL-1β-induced hyperresponsiveness to [Sar^9,$Met(O_2)^{11}$]-substance P (SP) (0.1 μM) in human isolated bronchi. □: control; ■: IL-1β; ▩: IgG (500 ng·mL^{-1}); ■: IL-1β + IgG (500 ng·mL^{-1}). Results are expressed as percentage of contraction *versus* acetylcholine (3 mM). Data are mean±SEM of n=8 experiments. NS: nonsignificant; *: $p<0.05$.

hyperresponsiveness of the human isolated bronchus to the tachykinin NK-1 receptor agonist [Sar^9,$Met(O_2)^{11}$]-SP *in vitro*.

This study was designed to investigate new mechanisms involved in previously reported AHR observed in inflammatory conditions created *in vitro* by the pro-inflammatory cytokine IL-1β [14]. In human airway cells in culture, IL-1β induced release of NGF from airway smooth muscle cells [16], epithelial cells [17, 18] and lung fibroblasts [15]. The current study demonstrated that IL-1β is also able to stimulate release of NGF from isolated ring segments from the human bronchus. Thus, overexpression of NGF may be due to increased release of NGF from bronchial structural cells, smooth muscle, epithelial cells and/or fibroblasts, upon stimulation by IL-1β. Acquisition of a secretory phenotype by the cells of human bronchi has been previously submitted as a new characteristic of airway cells actively participating in inflammation [15, 18, 20]. Additionally, increased release of NGF may also be derived from immune/inflammatory cells, such as lymphocytes, macrophages or mast cells [4] since these cells are also reported to secrete NGF [4, 21–23]. This suggests that increased NGF levels might occur in inflammatory conditions *in vivo*. Indeed, overexpression of NGF has been reported in humans *in vivo*, in situations where IL-1β is increased, particularly in asthma [24–27].

The present study also demonstrated that an anti-NGF blocking antibody abolishes the hyperresponsiveness to [Sar^9,$Met(O_2)^{11}$]-SP induced by IL-1β of the human bronchus *in vitro*, suggesting contribution of NGF to the development of AHR to IL-1β. Concordant suggestion has recently been reported in animal models, when administration of a blocking anti-NGF antibody prevents development of allergen-induced AHR to electrical field stimulation in mice [6] or attenuates the allergen-specific early airway response to ovalbumin in sensitised and challenged rats [28]. In addition, exogenous NGF has been reported to induce AHR by itself in the guinea-pig *in vivo* [9, 29], and tracheal hyperresponsiveness in the guinea-pig *in vitro* [10]. The present findings show that NGF also induces a dose-dependent AHR in the human isolated bronchus *in vitro*.

These results provide a potential mechanism to explain the findings of an upregulation of NGF protein in the airways of asthmatic patients, which has been associated with AHR, although no causal relationship between these events has been demonstrated. Indeed, enhanced NGF levels are reported in serum from patients with asthma, with the highest NGF levels in patients with more severe allergic asthma displaying a high degree of AHR [24]. Also, local upregulation of NGF protein was detected in bronchoalveolar lavage fluid from asthmatics, displaying AHR, as compared with control subjects [25]. These NGF levels have been further enhanced following allergen challenge in mild asthmatics [26]. In addition, previous data from KASSEL *et al.* [27] support an upregulation of NGF in the bronchi as an early event in response to allergen in asthma, in association with an increased AHR. Indeed, increased NGF transcripts and AHR were detected in the airway tissue from mild asthmatics following exposure to allergen at a low subclinical dose [27]. All these findings suggest a close association between increased NGF levels and AHR. The present results bring support to an underlying causal mechanism since they demonstrate that exogenous NGF is responsible for an increased responsiveness of the human bronchus, and that hyperresponsiveness induced by IL-1β is abolished by anti-NGF antibodies. In contrast, no increased contraction was observed in response to a maximal acetylcholine response. This is in agreement with previous findings in human isolated bronchi that IL-1β neither modifies acetylcholine maximal response nor displaced the acetylcholine concentration-response curve [14]. This may be linked to the reported heterogeneity of AHR to contractile agonists in asthma, which is now clearly established, but remains unexplained [30].

The mechanism by which NGF induces AHR is not known. NGF is reported to activate immune/inflammatory cells, such as mast cells, lymphocytes, basophils or macrophages, as well as structural resident cells, such as fibroblasts or airway smooth muscle cells [5]. NGF is also able to sensitise neurons, and it induces an enhanced production of tachykinins in sensory neurons. This was shown by HUNTER *et al.* [31], who demonstrated in the guinea-pig that NGF induces a phenotypic switch of airway sensory neurons 24 h after intratracheal administration, and that NGF increases SP content in neuronal cell bodies. Furthermore, HOYLE *et al.* [7] showed that transgenic mice overexpressing NGF in the airways develop a hyperinnervation of the airways, and an increase in SP content. However, these effects have been observed hours to days after NGF administration, involving transcriptional mechanisms that would probably not account for the rapid effect of NGF in the present experimental conditions. This evidence is further supported by the absence of sensory cell bodies within the preparation, indicating that upregulation of SP content cannot be a mechanism of NGF action in the human bronchus in these conditions *in vitro*. However, an early local mechanism may be proposed, as also observed in the hyperalgesia mechanism in the skin. Indeed, NGF, when applied directly to nociceptive afferents, lowers the threshold of thermal stimulation in an isolated skin-nerve preparation [32]. Similarly, NGF applied for 10 min on dorsal root ganglion neurons was able to abolish the tachyphylaxis observed in response to capsaicin or, even, to potentiate these responses [33]. Interestingly, NGF has also recently been shown in a model of inflammatory pain in the rat to have short-term effects on nociceptor sensitisation of sensory nerves of the dorsal root ganglia [34]. NGF might, therefore, contribute to an increased neuronal hyperexcitability, possibly through vanilloid receptor-1 activation without transcriptional events including their increased expression [35, 36]. These results, together with the present data, suggest that NGF is able to induce early post-transcriptional changes in neurons and cells, which may be involved in the tissue ''sensitisation'' [1, 37–39], and suggest parallel mechanisms in the generation of AHR and of hyperalgesia.

In conclusion, the current results clearly show that nerve growth factor released by interleukin-1β is involved in the airway hyperresponsiveness induced by this cytokine, and support the evidence of neuro-immune cross-talk in the hyperresponsiveness of the human bronchus.

REFERENCES

1 Levi-Montalcini R, Skaper SD, Dal Toso R, Petrelli L, Leon A. Nerve growth factor: from neurotrophin to neurokine. *Trends neurosci* 1996; 19: 514–520.
2 Aloe L, Bracci-Laudiero L, Bonini S, Manni L. The expanding role of nerve growth factor: from neurotrophic activity to immunologic diseases. *Allergy* 1997; 52: 883–894.
3 Bonini S, Lambiase A, Bonini S, Levi-Schaffer F, Aloe L. Nerve growth factor: an important molecule in allergic inflammation and tissue remodelling. *Int Arch Allergy Clin Immunol* 1999; 118: 159–162.
4 Olgart C, Frossard N. Nerve growth factor and asthma. *Pulm Pharm Ther* 2002; 15: 51–60.
5 Freund V, Frossard N. Expression of nerve growth factor in the airways and its possible role in asthma. *Prog Brain Res* 2004; 146: 335–346.
6 Braun A, Appel E, Baruch R, *et al.* Role of nerve growth factor in a mouse model of allergic inflammation and asthma. *Eur J Immunol* 1998; 28: 3240–3251.
7 Hoyle GW, Graham RM, Finkelstein JB, Nguyen K-PT, Gozal D, Friedman M. Hyperinnervation of the airways in transgenic mice overexpressing nerve growth factor. *Am J Respir Cell Mol Biol* 1998; 18: 149–157.
8 Päth G, Braun A, Meents N, *et al.* Augmentation of allergic early phase reaction to nerve growth factor. *Am J Respir Crit Care Med* 2002; 166: 818–826.
9 de Vries A, Dessing MC, Engels F, Henricks PAJ, Nijkamp FP. Nerve growth factor induces a neurokinin-1 receptor-mediated airway hyperresponsiveness in guinea pigs. *Am J Respir Crit Care Med* 1999; 159: 1541–1544.
10 de Vries A, Van Rijnsoever C, Engels F, Henricks PA, Nijkamp FP. The role of sensory nerve endings in nerve growth factor-induced airway hyperresponsiveness to histamine in guinea pigs. *Br J Pharmacol* 2001; 134: 771–776.
11 Van Oosterhout AJ, Nijkamp FP. Role of cytokines in bronchial hyperresponsiveness. *Pulm Pharmacol* 1993; 6: 225–236.
12 Tsukagoshi H, Sakamoto T, Xu W, Barnes PJ, Chung KF. Effect of interleukin-1 beta on airway hyperresponsiveness and inflammation in sensitized and nonsensitized Brown-Norway rats. *J Allergy Clin Immunol* 1994; 93: 464–469.
13 Molimard M, Naline E, Boichot E, *et al. In vitro* induced airway hyperresponsiveness to bradykinin. *Eur Respir J* 1998; 12: 1301–1306.
14 Barchasz E, Naline E, Molimard M, *et al.* Interleukin-1β-induced hyperresponsiveness to [Sar^9,$Met(O_2)^{11}$]-substance P in isolated human bronchi. *Eur J Pharmacol* 1999; 379: 87–95.
15 Olgart C, Frossard N. Human lung fibroblasts secrete nerve growth factor: effect of inflammatory cytokines and glucocorticoids. *Eur Respir J* 2001; 18: 115–221.
16 Freund V, Pons F, Joly V, Mathieu E, Martinet N, Frossard N. Upregulation of NGF expression by human airway smooth muscle cells in inflammatory conditions. *Eur Respir J* 2002; 20: 458–463.
17 Pons F, Freund V, Kuissu H, Mathieu E, Olgart C, Frossard N. Nerve growth factor secretion by human lung epithelial A549 cells in pro- and anti-inflammatory conditions. *Eur J Pharmacol* 2001; 428: 365–369.
18 Fox AJ, Patel HJ, Barnes PJ, Belvisi MG. Release of nerve growth factor by human pulmonary epithelial cells: role in airway inflammatory diseases. *Eur J Pharmacol* 2001; 424: 159–162.
19 Naline E, Molimard M, Regoli D, Emonds-Alt X, Bellamy JF, Advenier C. Evidence for functional tachykinin NK-1 receptors on human isolated small bronchi. *Am J Physiol* 1996; 271: 763–767.
20 Barnes PJ, Chung KF, Page CP. Inflammatory mediators in asthma: an update. *Pharmacol Rev* 1998; 50: 515–596.
21 Leon A, Buriani A, Dal Toso R, *et al.* Mast cells synthesize, store and release nerve growth factor. *Proc Nat Acad Sci* 1994; 91: 3739–3743.
22 Lambiase A, Bracci-Laudiero L, Bonini S, *et al.* Human CD4+ T cell clones produce and release nerve growth factor and express high affinity nerve growth factor receptors. *J Allergy Clin Immunol* 1997; 100: 408–414.
23 Skaper SD, Pollock M, Facci L. Mast cells differentially express and release active high molecular weight neurotrophins. *Molec Brain Res* 2001; 97: 177–185.
24 Bonini S, Lambiase A, Bonini S, *et al.* Circulating nerve growth factor levels are increased in human with allergic diseases and asthma. *Proc Natl Acad Sci USA* 1996; 93: 10955–10960.
25 Olgart Höglund C, de Blay F, Oster JP, *et al.* Nerve growth factor levels and localisation in human asthmatic bronchi. *Eur Respir J* 2002; 20: 1110–1116.
26 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.
27 Kassel O, de Blay F, Duvernelle C, *et al.* 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* 2001; 31: 1432–1440.
28 Glaab T, Hoymann HG, Hecht M, *et al.* Effects of anti-nerve growth factor on early and late airway responses in allergic rats. *Allergy* 2003; 58: 900–904.
29 Friberg SG, Olgart Höglund C, Gustafsson LE. Nerve growth factor increases airway responses and decreases levels of exhaled nitric oxide during histamine challenge in an *in vivo* guinea-pig model. *Acta Physiol Scand* 2001; 173: 239–245.
30 Van Schoor J, Joos GF, Pauwels RA. Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. *Eur Respir J* 2000; 16: 514–533.
31 Hunter DD, Myers AC, Undem BJ. Nerve growth factor-induced phenotypic switch in guinea-pig airway sensory neurons. *Am J Respir Crit Care Med* 2000; 161: 1985–1990.
32 Rueff A, Mendell LM. Nerve growth factor NT-5 induces increased thermal sensitivity of cutaneous nociceptors *in vitro*. *J Neurophysiol* 1996; 76: 3593–3596.
33 Shu XQ, Mendell LM. Neurotrophins and hyperalgesia. *Proc Nat Acad Sci USA* 1999; 96: 7693–7696.
34 Mamet J, Lazdunski M, Voilley N. How nerve growth factor drives physiological and inflammatory expressions of acid-sensing ion channel 3 in sensory neurons. *J Biol Chem* 2003; 278: 48907–48913.
35 Hu-Tsai M, Woolf C, Winter J. Influence of inflammation or disconnection from peripheral target tissue on the

capsaicin sensitivity of rat dorsal root ganglion sensory neurones. *Neurosci Lett* 1996; 19,203: 119–122.
36 Szallasi A, Blumberg PM. Vanilloid (capsaicin) receptors and mechanisms. *Pharmacological Rev* 1999; 51: 159–212.
37 Lewin GR, Rueff A, Mendell LM. Peripheral and central mechanisms of NGF-induced hyperalgesia. *Eur J Neurosci* 1994; 6: 1903–1912.
38 Nicholas RS, Winter J, Wren P, Bergmann R, Woolf CJ. Peripheral inflammation increases the capsaicin sensitivity of dorsal root ganglion neurons in a nerve growth factor-dependent manner. *Neurosci* 1999; 91: 1425–1433.
39 Woolf CJ, Costigan M. Transcriptional and posttranslational plasticity and the generation of inflammatory pain. *Proc Nat Acad Sci USA* 1999; 96: 7723–7730.