

REVIEW

The role of neurotrophins in allergic bronchial asthma

A. BRAUN, M. LOMMATZSCH and H. RENZ

Institut für Laboratoriumsmedizin und Pathobiochemie, Charité-Campus Virchow-Klinikum, Humboldt Universität, Berlin, Germany

Summary

Neurotrophins are known as target-derived factors regulating the development, survival and function of distinct neuronal subsets. This family of structurally related growth factors includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4. Beyond their originally described effects on neurones, neurotrophins are involved in many other biological processes including immune regulation, smooth muscle differentiation and neuroendocrine functions. The pathogenesis of allergic bronchial asthma is characterized by a complex interaction between inflammatory, epithelial, smooth muscle cells and neurones. This results in the development of airway inflammation, airway obstruction, airway hyperresponsiveness and airway remodelling. The altered neuronal control of airway smooth muscle tone is a hallmark in bronchial asthma. Growing evidence suggests that T cells, B cells, macrophages and mast cells produce high quantities of neurotrophic factors during allergic inflammation. Therefore, they are likely candidates as mediators between allergic inflammation and airway hyperresponsiveness. Neurotrophins increase excitability and neurotransmitter synthesis in sensory neurones and can also affect motor neurones. They enhance local neuropeptide release and augment central reflexes. Additionally, neurotrophins can affect immune functions and smooth muscle physiology. Therefore, neurotrophins are involved in the cellular communication between different compartments of the asthmatic lung. Delineating the mechanisms of this cross-talk will give new insights into the pathogenesis of asthma.

Neuronal changes in allergic bronchial asthma

Allergic bronchial asthma is a disease characterized by a defined type of airway inflammation, reversible airway obstruction in response to allergen inhalation and enhanced bronchoconstrictor responses to unspecific stimuli (airway

hyperresponsiveness, AHR). Repeated exposures to allergen result in the development of a TH2 immune response, the recruitment of eosinophils and lymphocytes into the airways, remodelling of airway epithelium, smooth muscle hypertrophy, basal membrane thickening and AHR [1–5]. In the last decade, growing evidence indicates neuronal dysregulation in allergic bronchial asthma [6]. Increased levels of the neuropeptide substance P have been detected in the lung of asthmatic patients [7] as well as in a mouse model [8]. Additionally, allergen challenge increased neurokinin A levels in bronchoalveolar lavage (BAL) fluid of asthmatic patients [9]. Substance P and neurokinin A mimic characteristic features of bronchial asthma including smooth muscle contraction, vascular dilatation, increased vascular permeability, mucus hypersecretion, facilitation of acetylcholine release and enhancement of inflammation (neurogenic inflammation) [10–12]. In humans, the bronchoconstriction is predominantly mediated by neurokinin A, whereas inflammatory effects are mediated by substance P [13]. In a guinea pig model, it has been shown that sensory innervation of the airways is altered by allergic inflammation [14,15]. The increase of substance P and neurokinin A in the lung in response to allergen challenge has been related to an increased production of these neuropeptides in neurones of the nodose ganglion [16]. Impaired degradation of tachykinins could further enhance their local activity [17]. Despite the existence of hard evidence supporting that c-fibre-released tachykinins play a role in human asthma, there are also studies based on capsaicin or antagonist treatment doubting a substantial role of tachykinins in bronchial asthma [18–20]. Therefore, the significance of tachykinins in the pathogenesis of human bronchial asthma remains to be elucidated using trials with specific tachykinin receptor antagonists.

Since cholinergic nerves represent the dominant bronchoconstrictor pathway and anticholinergic drugs are very effective bronchodilators in acute severe asthma, cholinergic mechanisms must be considered in the development of AHR. These mechanisms include enhanced cholinergic reflex activity, increased acetylcholine release, enhanced sensitivity of smooth muscle to acetylcholine and increased density of muscarinic receptors on airway smooth muscle

Correspondence: H. Renz, Clinical Chemistry and Molecular Diagnostics, Central Laboratory, University Hospital Baldingersh, 35033 Marburg, Germany.

[21]. Interestingly, cholinergic mechanisms were also shown to be affected by neuropeptides [22].

Tachykinins and inflammatory hypersensitivity

Substance P and neurokinin A are members of a family of closely related neuropeptides termed tachykinins. Tachykinins are encoded by the two *prae*protachykinin (PPT) genes PPT-A and PPT-B. They are synthesized preferentially in cell bodies of the sensory ganglia by a complex biosynthetic pathway [23]. These neuropeptides are transferred via axonal transport not only to presynaptic axon endings in the spinal cord, but also to peripheral sensory nerve endings [24,25]. Upon stimulation with mechanical, thermal, chemical (capsaicin, nicotine) or inflammatory stimuli (bradykinin, histamine, prostaglandins), tachykinins are released [10]. They act in a dual fashion, as afferent neurotransmitters to spinal cord motor neurones as well as efferent neurosecretory mediators diffusing into the peripheral tissue. In inflammatory processes, enhanced synthesis of tachykinins leads to inflammatory hypersensitivity. These effects are mediated by the neurotrophin nerve growth factor (NGF) in specific conditions [26–28]. Neurotrophins are very potent growth factors for lung-innervating sensory neurones [30]. Since these signalling molecules are locally upregulated in allergic inflammatory processes, they are candidates for mediating the neuronal changes occurring in allergic asthma.

The biology of neurotrophins and their receptors

The neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4) all belong to a family of homologous proteins that exert their effects primarily as target-derived, paracrine or autocrine neurotrophic factors. The role of the neurotrophins in survival, differentiation and maintenance of neurones is well defined [31]. They exhibit partially overlapping but distinct patterns of expression and cellular targets. In addition to their effects in the central nervous system (CNS), neurotrophins also affect peripheral afferent and efferent neurones. The biological effects of neurotrophins are mediated by binding either to high affinity ($K_d = 10^{-11}$) tyrosine kinase receptors (trkA, trkB, trkC) or the low affinity ($K_d = 10^{-9}$) pan-neurotrophin receptor (p75^{NTR}). Substantial biological effects of neurotrophins are mediated through the high affinity tyrosine kinase receptors. TrkA is the high affinity receptor for NGF, trkB for BDNF and NT-4, and trkC for NT-3. Neurotrophin receptors are widely expressed in the neurones of the peripheral and the central nervous system, both during development and in adults [31]. However, trk receptors as

well as p75^{NTR} are also expressed on non-neuronal cells including immune cells, muscle cells and epithelial cells.

Sources of neurotrophins in inflammation

The traditional cellular sources of neurotrophins under physiological conditions are primarily nerve-associated cells such as glia cells, Schwann cells or fibroblasts and neurones themselves [31,32]. In inflammatory processes, NGF is also produced by a wide range of haematopoietic cells including mast cells [33], macrophages [34], T cells [35] and B cells [36]. BDNF synthesis has been detected in activated human T cells, B cells and macrophages [37,38]; in mast cells [39] and in thrombocytes [40] (Table 1). In addition, neurones themselves produce enhanced levels of NGF and BDNF during inflammation and injury [41,42]. Elevated NGF levels have been described in several inflammatory conditions [43,44]. In allergic diseases including vernal keratoconjunctivitis and allergic rhinoconjunctivitis, a marked increase in NGF serum levels has been demonstrated [45]. Patients with severe allergic bronchial asthma display high serum levels of NGF as well [46].

To further characterize the cellular sources and the potential role of neurotrophins in allergic inflammation, we turned to an *in vivo* mouse model of bronchial asthma. In responses to aerosol allergen challenges, sensitized mice developed airway inflammation with high levels of TH2 cytokines, an influx of lymphocytes, neutrophils and eosinophils into the airways and airway hyperresponsiveness. In this condition of allergic inflammation, T cells, B cells and macrophages represent sources of enhanced NGF production [34]. *In vitro*, allergen stimulation of mononuclear cells from sensitized mice resulted in enhanced NGF synthesis. In addition, NGF production was enhanced by antigen stimulation in TH2 cell clones [47]. A different expression pattern was observed for BDNF. Beyond a constitutive production of BDNF by respiratory epithelial cells and airway smooth muscle cells [96], we have demonstrated that activated murine macrophages and T cells, but not B cells, produce BDNF [38]. Histological analysis of the inflamed lung revealed strong NGF and BDNF production in cells of the peribronchial inflammatory infiltrate [34,38].

Next, we evaluated the neurotrophin production in human bronchial asthma. For this purpose, we investigated the presence of NGF, BDNF and NT-3 in BAL after segmental allergen provocation in mild asthmatic patients. Neurotrophins increased markedly in allergen-exposed lung segments. Notably, this upregulation was seen during late-phase response, but not in the early phase [48]. Unfortunately, very limited information is available about the local production of neurotrophins in other inflammatory diseases. One report shows upregulation of NGF, BDNF and NT-3 synthesis in the experimentally inflamed bladder [49]. The cellular events and the signal

Table 1. Production of NGF and BDNF by immune cells

Cellular sources	Nerve growth factor (NGF)	Brain-derived neurotrophic factor (BDNF)
Mast cells	<i>In vitro</i> (human, rat) [33,39,88]	<i>In vitro</i> (rat) [39]
Macrophages	<i>In vivo</i> (mouse), in allergic airway inflammation, by activated, not resting macrophages [34]	<i>In vivo</i> (mouse), in allergic airway inflammation, by activated, not resting macrophages [38] <i>In vivo</i> (human), in inflammatory lesions of multiple sclerosis [37] <i>In vitro</i> (human), by activated, not resting macrophages [37]
T cells	<i>In vivo</i> (mouse) in allergic airway inflammation (unpublished data) <i>In vitro</i> (human), upregulated in activated TH2-clones [89] <i>In vitro</i> (mouse), by TH1 and TH2 clones, only enhanced in TH2-clones by antigen stimulation [47]	<i>In vivo</i> (mouse) in allergic airway inflammation [38] <i>In vitro</i> (human), enhanced following antigen stimulation [37]
B cells	<i>In vitro</i> (human/mouse), produced by activated B cells [36,47]	<i>In vitro</i> (human), enhanced following activation [37] <i>In vivo/in vitro</i> (mouse), not detectable in allergic airway inflammation [38]
Platelets	Not detectable [40]	By human, but not by murine platelets [40,90]
Eosinophils	Low production [91]	?

transduction pathways leading to the elevated neurotrophin synthesis in inflammation remain unknown.

Functions of neurotrophins in the peripheral nervous system

Since most of the sensory neurones innervating the lung are responsive to neurotrophins, and neurotrophins also affect functions of motor neurones, local production of neurotrophins in the lung could mediate characteristic neuronal changes in allergic bronchial asthma. It has been well established that visceral sensory neurones, localized in the nodose and dorsal root ganglia, require neurotrophins for survival during development [29,50,51]. Interestingly, the apoptosis of neuronal subpopulations in BDNF knockout

mice leads to severe deficits in breathing control [29]. In adults, functional properties of neurones are also affected by neurotrophins [52,53]. NGF was shown to upregulate neuropeptide production in sensory neurones and contribute to inflammatory hypersensitivity [27,54,55] (Fig. 1). In addition, neuronal hyperreflexia in cystitis was demonstrated to be mediated by NGF [28]. Though cultured nodose ganglion neurones do not require NGF for survival, their substance P production is regulated by NGF [56]. In NGF-overexpressing mice, a marked increase of neuropeptide production was observed in projecting sensory neurones [30,57]. There is growing evidence that BDNF modulates neuropeptide production not only in CNS neurones [58], but also in PNS neurones [59]. In addition, BDNF regulates capsaicin sensitivity in nodose ganglion neurones, whereas NGF exerts

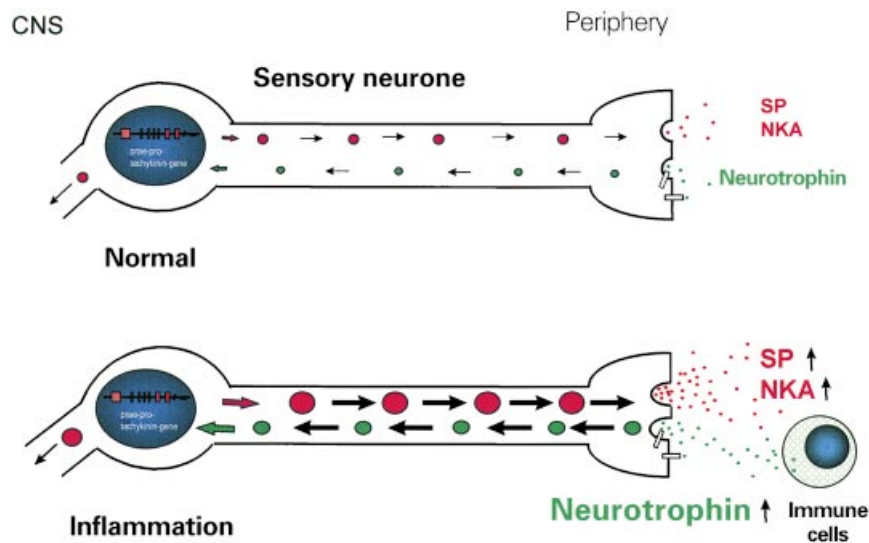


Fig. 1. Control of neuronal neuropeptide production by target-derived neurotrophins during inflammation. Under physiological conditions, low levels of neurotrophins are produced by peripheral innervation targets contributing to the maintenance and survival of neurones. During inflammation, immune cells such as T cells, B cells, macrophages and mast cells produce high quantities of neurotrophins. These neurotrophins are recognized by specific trk receptors, internalized and retrogradely transported to the neuronal cell bodies. In the nucleus they induce transcriptional activation leading to neuronal hyperreactivity and enhanced neuropeptide synthesis (SP: substance P, NKA: neurokinin A). These neuropeptides are transported into the periphery, and will be released upon stimulation

these effects primarily in dorsal root ganglia. Therefore, neurotrophins probably also affect the sensitivity of sensory neurones to certain stimuli [15,60]. Taken together, neurotrophins could orchestrate an enhanced neuropeptide release and increase in excitability of sensory neurones (Fig. 2). Actions of neurotrophins on visceral motor neurones are

less well defined. However, the presence of trk receptors on these neurones and the marked retrograde neurotrophin transport in viscerosfferences suggest neurotrophins could also act on efferent neurones [61]. Evidence implies that essential functions of the cholinergic system are also under neurotrophin control [62–64].

Fig. 2. The contribution of neurotrophins to neuronal changes in allergic bronchial asthma. Neurotrophins produced by immune cells in the inflamed lung induce neuropeptide production and nerve hyperreactivity in sensory c-fibres. Upon non-specific stimulation (cold air, cigarette smoke, etc.) increased amounts of neuropeptides can be antidromically released (axon reflex) causing smooth muscle contraction, vascular dilatation and increased vascular permeability, mucus hypersecretion, facilitation of acetylcholine release and enhancement of inflammation (neurogenic inflammation). In addition, neurotrophin-induced sensory hyperreactivity could augment local and central reflexes contributing to airway constriction

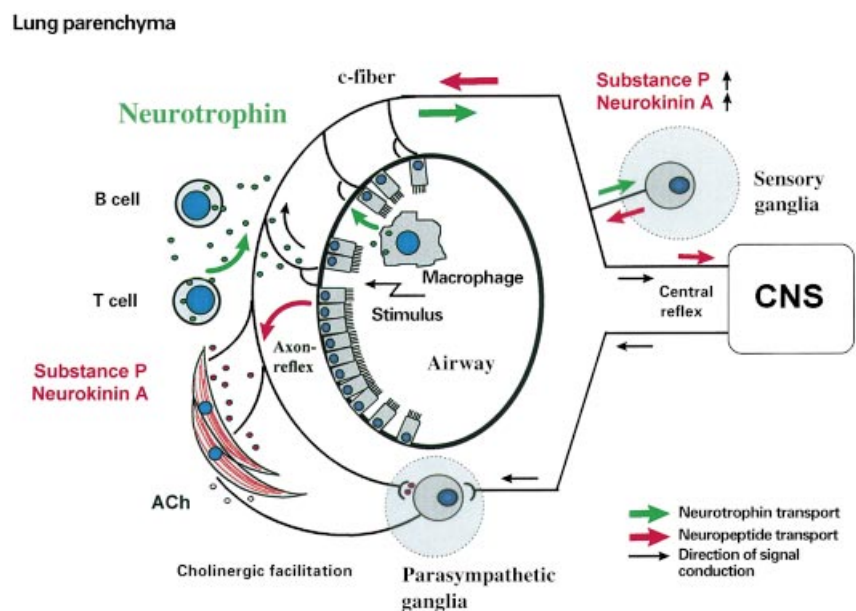


Table 2. Neurotrophin receptors on immune cells

Cell type	p 75	trkA	trkB	trkC	References
Mast cells	—	+	—	—	[88,92]
Macrophages	—	+	+	—	[65,68,72,93,94]
T cells	—	+	+/-	—	[35,36,67,68,93]
B cells	+	+	?	?	[36,95]
Eosinophils	?	?	?	?	

—, not detected, +, detected, +/-, conflicting data, ?, limited information

The functions of neurotrophins in the immune system

Beyond neurones, trkA and/or p75^{NTR} are also expressed on a variety of immune cells, including T cells, B cells, monocytes and mast cells [33,36,65,66]. TrkB was shown to be expressed on T cells and macrophages [67,68] (Table 2). NGF increases the number of mast cells in neonatal rats [69], stimulates rapid degranulation of mast cells and basophils [70,71], promotes differentiation of mast cells, granulocytes and macrophages [72–75], activates eosinophils [76], promotes proliferation of B- and T-cell subsets [77,78], enhances TH2 cytokine production and immunoglobulin (Ig) E synthesis [34] and induces differentiation of activated B cells in Ig-secreting plasma cells [79]. Summarizing, most of these NGF effects have pro-inflammatory properties. The majority of neurotrophin effects were observed only in activated or costimulated cells. NGF by itself does not appear to activate the immune cells in physiologically relevant concentrations, but rather modulates their threshold to other triggering stimuli [80]. However, most of these studies were performed *in vitro*. Therefore, the physiological function of neurotrophins *in vivo* remains to be elucidated. It must be noted that there is very little data available about possible functions of other neurotrophins in the immune system.

Functions of neurotrophins in smooth muscle physiology

Since the smooth muscle is one of the major effector organs in allergic asthma, direct effects of neurotrophins on smooth muscle cells must be considered. Smooth muscle cells are able to produce all neurotrophins as well as to express their receptors [81]. A role of NGF was postulated in myoblast differentiation and proliferation [82]. The upregulation of neurotrophin receptors in vascular smooth muscle cells has been shown following de-endothelialization [83] and incubation with serum [81]. Interestingly, smooth muscle cells markedly upregulate NGF and BDNF as well as trkA and trkB receptor expression after injury [83]. These data suggest that smooth muscle cells can produce and respond

to neurotrophins in inflammatory conditions. This leads to the hypothesis that neurotrophins could contribute to the altered smooth muscle function in bronchial asthma.

Neurotrophins and asthma

Neurotrophins are produced locally in allergic inflammation by immune cells. In addition, stress-related events both in animal models and in humans result in a systemic increase of NGF, suggesting this molecule is also involved in neuro-endocrine functions [84,85]. The rapid release of NGF is part of an alerting signal in response to either psychologically stressful or anxiogenic conditions [86,87]. Neurotrophins can contribute to the pathophysiological changes in asthma in many ways (Fig. 3). There is good evidence from mouse models of allergic inflammation and from NGF transgenic mice that neurotrophins alter sensory innervation, enhance neuropeptide production and induce airway hyperresponsiveness [30,34]. In these models, airway hyperresponsiveness was induced by neurotrophins independently of inflammation. The increase of neurotrophins in BAL fluid after segmental allergen provocation [48] indicates similar neuronal mechanisms in human asthma. Furthermore, other recently discovered effects of neurotrophins must be considered in the pathogenesis of asthma.

Conclusion

Neurotrophins could participate in the pathogenesis of bronchial asthma in several ways: (1) as nerve growth

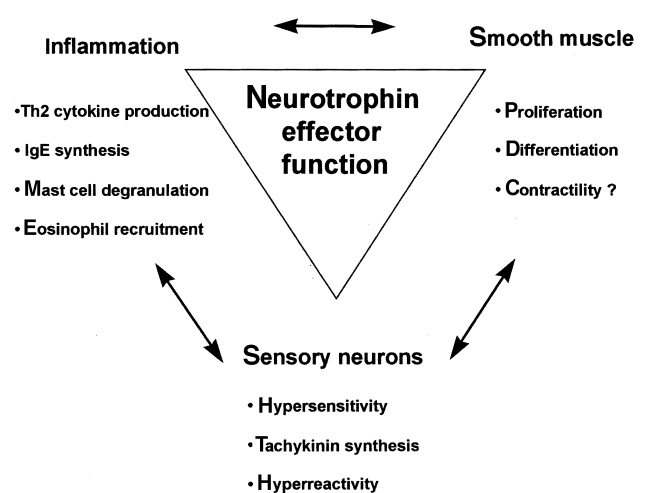


Fig. 3. The effects of neurotrophins in allergic asthma. Neurotrophins can act on several cells involved in the pathogenesis of allergic bronchial asthma. They have pro-inflammatory properties on immune cells, induce neuronal hyperreactivity and contribute to smooth muscle proliferation

factors inducing neuropeptide synthesis and neuronal hyperreactivity, (2) as cytokine-like factors influencing the allergic immune response and inflammation, and (3) as growth factors directly controlling smooth muscle function. There is growing evidence for a particular role of NGF and BDNF in this condition. By contrast, the functional significance of NT-3 and NT-4 remains enigmatic. Since the pathogenesis of allergic bronchial asthma is characterized by a complex interaction between inflammatory cells, epithelia, smooth muscle cells and neurones in the lung, neurotrophins could play a critical role in this pulmonary cross-talk.

Acknowledgements

This work was supported by the Volkswagen Stiftung. This work is dedicated to the 60th birthday of Professor Eckart Kötting.

References

- Hogan SP, Mould A, Kikutani H, Ramsay AJ, Foster PS. Aeroallergen-induced eosinophilic inflammation, lung damage, and airways hyperreactivity in mice can occur independently of IL-4 and allergen-specific immunoglobulins. *J Clin Invest* 1997; 99:1329–39.
- Lambrecht BN, Salomon B, Klatzmann D, Pauwels RA. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J Immunol* 1998; 160:4090–7.
- Blyth DI, Pedrick MS, Savage TJ, Bright H, Beesley JE, Sanjar S. Induction, duration, and resolution of airway goblet cell hyperplasia in a murine model of atopic asthma: effect of concurrent infection with respiratory syncytial virus and response to dexamethasone. *Am J Respir Cell Mol Biol* 1998; 19:38–54.
- Kay AB. Pathology of mild, severe, and fatal asthma. *Am J Respir Crit Care Med* 1996; 154:S66–9.
- Virchow JC Jr, Walker C, Hafner D, Kortsik C, Werner P, Matthys H, Kroegel C. T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. *Am J Respir Crit Care Med* 1995; 151:960–8.
- Lundberg JM. Tachykinins, sensory nerves, and asthma – an overview. *Can J Physiol Pharmacol* 1995; 73:908–14.
- Baumgarten CR, Witzel A, Kleine Tebbe J, Kunkel G. Substance P enhances antigen-evoked mediator release from human nasal mucosa. *Peptides* 1996; 17:25–30.
- Kaltreider HB, Ichikawa S, Byrd PK et al. Upregulation of neuropeptides and neuropeptide receptors in a murine model of immune inflammation in lung parenchyma. *Am J Respir Cell Mol Biol* 1997; 16:133–44.
- Heaney LG, Cross LJ, McGarvey LP, Buchanan KD, Ennis M, Shaw C. Neurokinin A is the predominant tachykinin in human bronchoalveolar lavage fluid in normal and asthmatic subjects. *Thorax* 1998; 53:357–62.
- Barnes PJ. Neuroeffector mechanisms: the interface between inflammation and neuronal responses. *J Allergy Clin Immunol* 1996; 98:S73–81.
- Cheung D, van der Veen H, den Hartigh J, Dijkman JH, Sterk PJ. Effects of inhaled substance P on airway responsiveness to methacholine in asthmatic subjects in vivo. *J Appl Physiol* 1994; 77:1325–32.
- Cheung D, Timmers MC, Zwinderman AH, den Hartigh J, Dijkman JH, Sterk PJ. Neutral endopeptidase activity and airway hyperresponsiveness to neurokinin A in asthmatic subjects in vivo. *Am Rev Respir Dis* 1993; 148:1467–73.
- Joos G, Pauwels R, van der Straeten M. Effect of inhaled substance P and neurokinin A on the airways of normal and asthmatic subjects. *Thorax* 1987; 42:779–83.
- Riccio MM, Proud D, Undem BJ. Enhancement of afferent nerve excitability in the airways by allergic inflammation. *Pulm Pharmacol* 1995; 8:181–5.
- Undem BJ, Hunter DD, Liu M, Haak-Frendscho M, Oakragly A, Fischer A. Allergen-induced sensory neuroplasticity in airways. *Int Arch Allergy Immunol* 1999; 118:150–3.
- Fischer A, McGregor GP, Saria A, Philippin B, Kummer W. Induction of tachykinin gene and peptide expression in guinea pig nodose primary afferent neurons by allergic airway inflammation. *J Clin Invest* 1996; 98:2284–91.
- van der Velden VHJ, Hulsmann AR. Peptidases: structure, function and modulation of peptide-mediated effects in the human lung. *Clin Exp Allergy* 1999; 29:445–56.
- Reynolds PN, Holmes MD, Scicchitano R. Role of tachykinins in bronchial hyper-responsiveness. *Clin Exp Pharmacol Physiol* 1997; 24:273–80.
- Van Schoor J, Joos GF, Chasson BL, Brouard RJ, Pauwels RA. The effect of the NK2 tachykinin receptor antagonist SR 48968 (saredutant) on neurokinin A-induced bronchoconstriction in asthmatics. *Eur Respir J* 1998; 12:17–23.
- Ellis JL, Sham JS, Undem BJ. Tachykinin-independent effects of capsaicin on smooth muscle in human isolated bronchi. *Am J Respir Crit Care Med* 1997; 155:751–5.
- Casale TB. *Neurogenic Control of Inflammation and Airway Function*. St Louis, MO: Mosby, 1996.
- Mackay TW, Hulks G, Douglas NJ. Non-adrenergic, non-cholinergic function in the human airway. *Respir Med* 1998; 92:461–6.
- Nawa H, Kotani H, Nakanishi S et al. Tissue-specific generation of two preprotachykinin mRNAs from one gene by alternative RNA splicing. *Nature* 1984; 312:729–34.
- Hokfelt T, Kellerth JO, Nilsson G, Pernow B. Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons. *Brain Res* 1975; 100:235–52.
- Brimijoin S, Lundberg JM, Brodin E, Hokfelt T, Nilsson G. Axonal transport of substance P in the vagus and sciatic nerves of the guinea pig. *Brain Res* 1980; 191:443–57.
- Woolf CJ, Safieh Garabedian B, Ma QP, Crilly P, Winter J. Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. *Neuroscience* 1994; 62:327–31.
- Donnerer J, Schuligoi R, Stein C. 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* 1992; 49:693–8.
- 28 Dmitrieva N, Shelton D, Rice AS, McMahon SB. The role of nerve growth factor in a model of visceral inflammation. *Neuroscience* 1997; 78:449–59.
 - 29 Erickson JT, Conover JC, Borday V et al. Mice lacking brain-derived neurotrophic factor exhibit visceral sensory neuron losses distinct from mice lacking NT4 and display a severe developmental deficit in control of breathing. *J Neurosci* 1996; 16:5361–71.
 - 30 Hoyle GW, Graham RM, Finkelstein JB, Nguyen KPT, Gozal D, Friedman M. Hyperinnervation of the airways in transgenic mice overexpressing nerve growth factor. *Am J Respir Cell Mol Biol* 1998; 18:149–57.
 - 31 Lewin GR, Barde YA. Physiology of the neurotrophins. *Annu Rev Neurosci* 1996; 19:289–317.
 - 32 Levi Montalcini R, Dal Toso R, della Valle F, Skaper SD, Leon A. Update of the NGF saga. *J Neurol Sci* 1995; 130:119–27.
 - 33 Leon A, Buriani A, Dal Toso R et al. Mast cells synthesize, store, and release nerve growth factor. *Proc Natl Acad Sci U S A* 1994; 91:3739–43.
 - 34 Braun A, Appel E, Baruch R et al. Role of nerve growth factor in a mouse model of allergic airway inflammation and asthma. *Eur J Immunol* 1998; 28:3240–51.
 - 35 Ehrhard PB, Erb P, Graumann U, Otten U. Expression of nerve growth factor and nerve growth factor receptor tyrosine kinase Trk in activated CD4-positive T-cell clones. *Proc Natl Acad Sci U S A* 1993; 90:10984–8.
 - 36 Torcia M, Bracci Laudiero L, Lucibello M et al. Nerve growth factor is an autocrine survival factor for memory B lymphocytes. *Cell* 1996; 85:345–56.
 - 37 Kerschensteiner M, Gallmeier E, Behrens L et al. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? *J Exp Med* 1999; 189:865–70.
 - 38 Braun A, Lommatzsch M, Mannsfeldt A et al. Cellular sources of enhanced brain-derived neurotrophic factor production in a mouse model of allergic inflammation. *Am J Respir Cell Mol Biol* 1999; 21:537–46.
 - 39 Tam SY, Tsai M, Yamaguchi M, Yano K, Butterfield JH, Galli SJ. Expression of functional TrkA receptor tyrosine kinase in the HMC-1 human mast cell line and in human mast cells. *Blood* 1997; 90:1807–20.
 - 40 Yamamoto H, Gurney ME. Human platelets contain brain-derived neurotrophic factor. *J Neurosci* 1990; 10:3469–78.
 - 41 Cho HJ, Kim SY, Park MJ, Kim DS, Kim JK, Chu MY. Expression of mRNA for brain-derived neurotrophic factor in the dorsal root ganglion following peripheral inflammation. *Brain Res* 1997; 749:358–62.
 - 42 Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H. 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* 1992; 119:45–54.
 - 43 Bracci Laudiero LB, Aloe L, Levi Montalcini R et al. Multiple sclerosis patients express increased levels of beta-nerve growth factor in cerebrospinal fluid. *Neurosci Lett* 1992; 147:9–12.
 - 44 Bracci Laudiero L, Aloe L, Levi Montalcini R et al. Increased levels of NGF in sera of systemic lupus erythematosus patients. *Neuroreport* 1993; 4:563–5.
 - 45 Lambiase A, Bonini S, Bonini S et al. Increased plasma levels of nerve growth factor in vernal keratoconjunctivitis and relationship to conjunctival mast cells. *Invest Ophthalmol Vis Sci* 1995; 36:2127–32.
 - 46 Bonini S, Lambiase A, Bonini S et al. Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc Natl Acad Sci U S A* 1996; 93:10955–60.
 - 47 Santambrogio L, Benedetti M, Chao MV et al. Nerve growth factor production by lymphocytes. *J Immunol* 1994; 153:4488–95.
 - 48 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; 158:2002–5.
 - 49 Oddia D, Anand P, McMahon SB, Rattray M. Rapid increase of NGF, BDNF and NT-3 mRNAs in inflamed bladder. *Neuroreport* 1998; 9:1455–8.
 - 50 Wetmore C, Olson L. Neuronal and nonneuronal expression of neurotrophins and their receptors in sensory and sympathetic ganglia suggest new intercellular trophic interactions. *J Comp Neurol* 1995; 353:143–59.
 - 51 Snider WD. Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* 1994; 77:627–38.
 - 52 Chalazonitis A, Peterson ER, Crain SM. Nerve growth factor regulates the action potential duration of mature sensory neurons. *Proc Natl Acad Sci U S A* 1987; 84:289–93.
 - 53 Mandelzys A, Cooper E, Verge VM, Richardson PM. Nerve growth factor induces functional nicotinic acetylcholine receptors on rat sensory neurons in culture. *Neuroscience* 1990; 37:523–30.
 - 54 Lindsay RM, Harnmar AJ. Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. *Nature* 1989; 337:362–4.
 - 55 Vedder H, Affolter HU, Otten U. Nerve growth factor (NGF) regulates tachykinin gene expression and biosynthesis in rat sensory neurons during early postnatal development. *Neuropeptides* 1993; 24:351–7.
 - 56 MacLean DB, Lewis SF, Wheeler FB. Substance P content in cultured neonatal rat vagal sensory neurons: the effect of nerve growth factor. *Brain Res* 1988; 457:53–62.
 - 57 Albers KM, Wright DE, Davis BM. Overexpression of nerve growth factor in epidermis of transgenic mice causes hypertrophy of the peripheral nervous system. *J Neurosci* 1994; 14:1422–32.
 - 58 Nawa H, Pelleymounter MA, Carnahan J. Intraventricular administration of BDNF increases neuropeptide expression in newborn rat brain. *J Neurosci* 1994; 14:3751–65.
 - 59 Yao L, Zhang D, Bernd P. Differential regulation of substance P by all members of the nerve growth factor family of

- neurotrophins in avian dorsal root ganglia throughout development. *Neuroscience* 1997; 79:1197–206.
- 60 Winter J. Brain derived neurotrophic factor, but not nerve growth factor, regulates capsaicin sensitivity of rat vagal ganglion neurones. *Neurosci Lett* 1998; 241:21–4.
 - 61 Helke CJ, Adryan KM, Fedorowicz J et al. Axonal transport of neurotrophins by visceral afferent and efferent neurons of the vagus nerve of the rat. *J Comp Neurol* 1998; 393:102–17.
 - 62 Fu AK, Ip FC, Lai KO, Tsim KW, Ip NY. Muscle-derived neurotrophin-3 increases the aggregation of acetylcholine receptors in neuron-muscle co-cultures. *Neuroreport* 1997; 8:3895–900.
 - 63 Gonzalez M, Collins WF. Modulation of motoneuron excitability by brain-derived neurotrophic factor. *J Neurophysiol* 1997; 77:502–6.
 - 64 Fernandes KJ, Kobayashi NR, Jasmin BJ, Tetzlaff W. Acetylcholinesterase gene expression in axotomized rat facial motoneurons is differentially regulated by neurotrophins: correlation with trkB and trkC mRNA levels and isoforms. *J Neurosci* 1998; 18:9936–47.
 - 65 Ehrhard PB, Ganter U, Stalder A, Bauer J, Otten U. Expression of functional trk protooncogene in human monocytes. *Proc Natl Acad Sci U S A* 1993; 90:5423–7.
 - 66 Ehrhard PB, Erb P, Graumann U, Schmutz B, Otten U. Expression of functional trk tyrosine kinase receptors after T cell activation. *J Immunol* 1994; 152:2705–9.
 - 67 Maroder M, Bellavia D, Meco D et al. Expression of trkB neurotrophin receptor during T cell development. Role of brain derived neurotrophic factor in immature thymocyte survival. *J Immunol* 1996; 157:2864–72.
 - 68 Garcia Suarez O, Hannestad J, Esteban I, Sainz R, Naves FJ, Vega JA. Expression of the TrkB neurotrophin receptor by thymic macrophages. *Immunology* 1998; 94:235–41.
 - 69 Aloe L, Levi Montalcini R. Mast cells increase in tissues of neonatal rats injected with the nerve growth factor. *Brain Res* 1977; 133:358–66.
 - 70 Horigome K, Bullock ED, Johnson EM Jr. Effects of nerve growth factor on rat peritoneal mast cells. Survival promotion and immediate-early gene induction. *J Biol Chem* 1994; 269:2695–702.
 - 71 Bischoff SC, Dahinden CA. Effect of nerve growth factor on the release of inflammatory mediators by mature human basophils. *Blood* 1992; 79:2662–9.
 - 72 Susaki Y, Shimizu S, Katakura K et al. Functional properties of murine macrophages promoted by nerve growth factor. *Blood* 1996; 88:4630–7.
 - 73 Kannan Y, Matsuda H, Ushio H, Kawamoto K, Shimada Y. Murine granulocyte-macrophage and mast cell colony formation promoted by nerve growth factor. *Int Arch Allergy Immunol* 1993; 102:362–7.
 - 74 Matsuda H, Kannan Y, Ushio H et al. Nerve growth factor induces development of connective tissue-type mast cells in vitro from murine bone marrow cells. *J Exp Med* 1991; 174:7–14.
 - 75 Welker P, Grabbe J, Grutzkau A, Henz BM. Effects of nerve growth factor (NGF) and other fibroblast-derived growth factors on immature human mast cells (HMC-1). *Immunology* 1998; 94:310–7.
 - 76 Hamada A, Watanabe N, Ohtomo H, Matsuda H. Nerve growth factor enhances survival and cytotoxic activity of human eosinophils. *Br J Haematol* 1996; 93:299–302.
 - 77 Otten U, Ehrhard P, Peck R. Nerve growth factor induces growth and differentiation of human B lymphocytes. *Proc Natl Acad Sci U S A* 1989; 86:10059–63.
 - 78 Thorpe LW, Perez Polo JR. The influence of nerve growth factor on the in vitro proliferative response of rat spleen lymphocytes. *J Neurosci Res* 1987; 18:134–9.
 - 79 Brodie C, Gelfand EW. Regulation of immunoglobulin production by nerve growth factor: comparison with anti-CD40. *J Neuroimmunol* 1994; 52:87–96.
 - 80 Levi Montalcini R, Skaper SD, Dal Toso R, Petrelli L, Leon A. Nerve growth factor: from neurotrophin to neurokine. *Trends Neurosci* 1996; 19:514–20.
 - 81 Nemoto K, Fukamachi K, Nemoto F et al. Gene expression of neurotrophins and their receptors in cultured rat vascular smooth muscle cells. *Biochem Biophys Res Com* 1998; 245:284–8.
 - 82 Seidl K, Erck C, Buchberger A. Evidence for the participation of nerve growth factor and its low-affinity receptor (p75NTR) in the regulation of the myogenic program. *J Cell Physiol* 1998; 176:10–21.
 - 83 Donovan MJ, Miranda RC, Kraemer R et al. Neurotrophin and neurotrophin receptors in vascular smooth muscle cells. Regulation of expression in response to injury. *Am J Pathol* 1995; 147:309–24.
 - 84 Ojeda SR, Hill DF, Katz KH. The genes encoding nerve growth factor and its receptor are expressed in the developing female rat hypothalamus. *Brain Res Mol Brain Res* 1991; 9:47–55.
 - 85 Lahtinen T, Soinila S, Lakshmanan J. Biological demonstration of nerve growth factor in the rat pituitary gland. *Neuroscience* 1989; 30:165–70.
 - 86 Aloe L, Bracci Laudiero L, Alleve E, Lambiase A, Micera A, Tirassa P. Emotional stress induced by parachute jumping enhances blood nerve growth factor levels and the distribution of nerve growth factor receptors in lymphocytes. *Proc Natl Acad Sci U S A* 1994; 91:10440–4.
 - 87 Missale C, Boroni F, Sigala S et al. Nerve growth factor in the anterior pituitary: localization in mammothroph cells and cosecretion with prolactin by a dopamine-regulated mechanism. *Proc Natl Acad Sci U S A* 1996; 93:4240–5.
 - 88 Nilsson G, Forsberg Nilsson K, Xiang Z, Hallbook F, Nilsson K, Metcalfe DD. Human mast cells express functional TrkA and are a source of nerve growth factor. *Eur J Immunol* 1997; 27:2295–301.
 - 89 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; 100:408–14.
 - 90 Radka SF, Holst PA, Fritsche M, Altar CA. Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. *Brain Res* 1996; 709:122–301.

- 91 Solomon A, Aloe L, Pe'er J et al. Nerve growth factor is preformed in and activates human peripheral blood eosinophils. *J Allergy Clin Immunol* 1998; 102:454–60.
- 92 Horigome K, Pryor JC, Bullock ED, Johnson EM Jr. Mediator release from mast cells by nerve growth factor. Neurotrophin specificity and receptor mediation. *J Biol Chem* 1993; 268:14881–7.
- 93 Garcia Suarez O, Hannestad J, Esteban I, Del Valle MM, Naves FJ, Vega JA. Neurotrophin receptor-like protein immunoreactivity in human lymph nodes. *Anat Rec* 1997; 249:226–32.
- 94 Shibayama E, Koizumi H. Cellular localization of the Trk neurotrophin receptor family in human non-neuronal tissues. *Am J Pathol* 1996; 148:1807–18.
- 95 Melamed I, Kelleher CA, Franklin RA et al. Nerve growth factor signal transduction in human B lymphocytes is mediated by gp140trk. *Eur J Immunol* 1996; 26:1985–92.
- 96 Lommatzsch M, Braun A, Mannsfeldt A et al. Abundant production of BDNF by adult visceral epithelia: implications for paracrine and target-derived neurotrophic functions. *Am J Pathol* 1999; 155:1183–93.