The contribution of neurotrophins to the pathogenesis of allergic asthma

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Abstract
The neurotrophins nerve growth factor, brain-derived neurotrophic factor, NT-3 (neurotrophin 3) and NT-4 are known for regulating neuron development, function and survival. Beyond this, neurotrophins were found to exert multiple effects on non-neuronal cells such as immune cells, smooth muscle and epithelial cells. In allergic asthma, airway inflammation, airway obstruction, AHR (airway hyperresponsiveness) and airway remodelling are characteristic features, indicating an intensive interaction between neuronal, structural and immune cells in the lung. In allergic asthma patients, elevated neurotrophin levels in the blood and locally in the lung are commonly observed. Additionally, structural cells of the lung and immune cells, present in the lung during airway inflammation, were shown to be capable of neurotrophin production. A functional relationship between neurotrophins and the main features of asthma was revealed, as airway obstruction, airway inflammation, AHR and airway remodelling were all shown to be stimulated by neurotrophins. The aim of the present review is to provide an overview of neurotrophin sources and target cells in the lung, concerning their possible role as mediators between structural cells, immune cells and neurons, connecting the different features of allergic asthma.

Introduction
Asthma is a chronic disorder of the airways that is characterized by reversible airflow obstruction in response to allergen, airway inflammation, persistent AHR (airway hyperresponsiveness) and airway remodelling. The aetiology of asthma is complex and multifactorial. Recent advances have demonstrated the importance of genetics and environmental influences in the development of asthma. Research activities of the last few years focused on an understanding of allergic airway inflammation. Allergic airway inflammation is characterized by an influx of lymphocytes, monocytes and eosinophils into the lung and this is associated with the presence of a Th2 (T helper cell type 2) type immune response and elevated IgE levels in serum. In previous studies, it has been shown that goblet cell hyperplasia, increased mucus production and AHR are associated with airway inflammation [1]. Enhanced levels of neurotrophins were observed in sera [2] and BALF (bronchoalveolar lavage fluid) of patients with allergic asthma [3], which were further enhanced after allergen provocation [4]. The aim of the present review is to elucidate the pleiotropic role of neurotrophins in the pathogenesis of allergic asthma.

Neurotrophins and their receptors
Neurotrophins were originally described as molecules regulating neurogenesis, neuronal differentiation and survival in the central and peripheral nervous system. NGF (nerve growth factor) represents the first isolated and best-characterized member of the neurotrophin family and was discovered by Levi-Montalcini and Hamburger [5] in the 1950s. Nowadays, the family of neurotrophins consists of NGF and the structurally and functionally similar polypeptides BDNF (brain-derived neurotrophic factor), NT-3 (neurotrophin 3) and NT-4. Neurotrophins are synthesized as preproproteins and are subsequently cleaved to smaller active peptides. They exert their function by binding two different receptor subtypes. The low-affinity pan-neurotrophin receptor p75NTR belongs to the TNF (tumour necrosis factor) receptor family, whereas the Trks (tropomyosin receptor kinases) TrkA, TrkB and TrkC are tyrosine kinases. In contrast with p75NTR, which binds all neurotrophins, the Trk receptors exhibit ligand selectivity. NGF preferentially binds to p75NTR, which binds all neurotrophins, the Trk receptors exhibit ligand selectivity. NGF preferentially binds to TrkA, BDNF and NT-4 to TrkB, and TrkC is the preferred receptor for NT-3 [6].

Neurotrophins in asthma
The first data indicating an involvement of NGF in allergic diseases came from Aloe et al. [7]. When they measured NGF levels in serum of soldiers after parachute jumping, they recognized that one young soldier who had an allergy attack the day before had the highest NGF levels in serum [7]. A subsequent study showed that NGF levels were generally increased in allergic patients and that the amount of NGF was positively correlated with the severity of the...
disease, with the highest levels seen in asthma patients [2]. Later studies revealed that the BDNF serum levels are also enhanced in asthmatics [8]. Based on these findings, the role of neurotrophins in the pathogenesis of allergies became a focus of interest. BALF from asthmatic patients showed increased NGF levels [3], and this was further increased by SAP (segmental allergen provocation) [4]. After allergen challenge, NGF, BDNF and NT-3 levels in BALF were increased. These observations were confirmed in mouse models of allergic asthma, as allergen-challenged mice had an increase in NGF and BDNF levels in BALF [9,10]. The comparable effects of allergen challenge on neurotrophin production in humans and mice made it possible to use murine asthma models to investigate the cellular sources and also the target cells of neurotrophins in the lung.

**Cellular sources of neurotrophins in the lung**

**Resident lung cells**

The airway epithelium acts as a barrier between pulmonary lumen and the underlying tissue and constitutively expresses NGF [11], BDNF [12] and NT-3, with BDNF showing the highest levels of expression [13]. Enhanced epithelial BDNF production during airway inflammation has been shown in murine asthma models [10]. Pulmonary fibroblasts are another source of neurotrophins, as isolated human lung fibroblasts were shown to produce NGF [14]. Inflammatory cytokines such as TNFα and IL-1β ( interleukin 1β) are capable of enhancing epithelial and fibroblast neurotrophin production, as shown in a human airway epithelial cell line and isolated human lung fibroblasts [11,14]. Bronchial smooth-muscle cells constitutively express NGF, BDNF and NT-3 at similar levels, as revealed by immunohistochemistry of lung tissue from non-asthmatics [13]. In cultured human bronchial smooth-muscle cells, the production of NGF and BDNF has been shown to be influenced differentially by inflammatory cytokines. IL-1β caused an increase in NGF and BDNF production, whereas IFN-γ up-regulated NGF but down-regulated BDNF secretion [15]. TNFα is known to play a pivotal role in the pathogenesis of asthma in murine models, as anti-TNFα antibody treatment reduced airway inflammation and hyperresponsiveness to metacholine [16]. IL-1β also seems to be a potent asthma inducer, since mice treated with IL-1β developed many features of asthma, and inhibiting IL-1β signalling reduces AHR and airway inflammation [17,18]. These effects might be mediated in part by the enhanced production of neurotrophins. Evidence for this mechanism is provided by a study showing that IL-1β stimulated both NGF secretion and hyperresponsiveness to an NK-1 (neurokinin-1) receptor agonist in isolated bronchi. The IL-1β-induced hyperresponsiveness was abolished by treatment with anti-NGF antibodies, confirming a strong association between the presence of inflammatory cytokines, neurotrophins and AHR [19].

**Immune cells**

Immune cells can not only affect neurotrophin production of the resident lung cells by their secreted cytokines, but also are capable of producing neurotrophins themselves.

In allergic patients, lung resident mast cells express allergen-specific IgE on their surfaces. Upon renewed allergen contact, degranulation and mediator release occur, resulting in acute broncho-obstruction [EAR (early allergic response)]. Mast cells are able to produce the neurotrophins NGF, BDNF and NT-3, and IgE cross-linking on the surface of mast cells can also induce NGF release [20–22]. Thus allergen-induced neurotrophin release from mast cells may occur during allergic asthma, contributing to the observed elevated neurotrophin levels. However, Virchow et al. [4] were not able to detect elevated neurotrophin levels in bronchoalveolar lavage in early phase response, whereas neurotrophin levels were increased during late phase response, which is characterized by airway narrowing and infiltration of the lung with immune cells.

Two types of macrophages are resident in the lung, acting as sentinels for pathogens together with dendritic cells. Pulmonary macrophages show different neurotrophin expression patterns as studied in murine asthma models. Interstitial and alveolar macrophages both produce NGF upon allergen stimulation [4]. BDNF is constitutively expressed in interstitial macrophages, but alveolar macrophages instead produce BDNF only after activation [9,23]. In contrast, constitutive expression of NT-3 occurs only in alveolar macrophages, whereas NT-4 is present in both cell types [23]. Controversial results in surgical samples of human lung show only NGF but not BDNF or NT-3 expression in both interstitial and alveolar macrophages [13]. Human peripheral blood monocytes, precursors for pulmonary macrophages and dendritic cells, constitutively secrete BDNF, enhanced by TNFα and IL-6 [24]. Thus airway inflammation may not only induce monocyte infiltration but also enhance neurotrophin production.

Eosinophils, the most abundant cell type in the inflammatory infiltrate during late phase reaction in allergic asthma, are also able to produce neurotrophins. Isolated human peripheral blood eosinophils were found to produce and release NGF and NT-3 constitutively, and NGF but not NT-3 production was augmented by immune complex binding [25,26]. Recent results show that BDNF is also produced by eosinophils [27].

Another source of neurotrophins are lymphocytes, which mediate an adaptive immune response to the inhaled allergen. Controversial results were obtained concerning NGF production in T-cells. In one study, NGF production in both CD4+ helper T-cells and CD8+ effector T-cells could be detected, and NGF secretion was increased upon stimulation in Th2 but not Th1 cells [28], results that match the observed Th2-type immune response during allergic asthma. Other groups could only show NGF expression in CD4+ T-cells, but not in CD8+ [29]. Moreover, in vitro studies revealed constitutive BDNF expression in peripheral blood CD4+ and CD8+ T-cells that was further enhanced after antigen
stimulation [30]. These results may reflect that the Th2-type immune response during allergic asthma is augmenting neurotrophin production.

B-cells were also found to produce NGF and BDNF [28,30,31]. A recent study suggests an even more important role of B-cells rather than T-cells in producing NGF, BDNF and NT-3 upon antigen stimulation [32].

As there is massive infiltration of cells, especially eosinophils, into the lung during airway inflammation, the invading immune cells seem to be one main cellular source of neurotrophins during allergic asthma.

Functional relevance of neurotrophins in allergic bronchial asthma

Neurotrophins and allergen sensitization
As most of the immune cells are capable of producing neurotrophins as well as their receptors, it is very likely that allergen sensitization is influenced by neurotrophins.

Allergens are first recognized by dendritic cells, patrolling for pathogens in the lung tissue. As they encounter antigen, they become activated and travel to the draining lymph nodes, maturing during this migration. In the lymph node, antigen presentation to B- and T-cells induces clonal proliferation of antigen-specific lymphocytes and antibody production. In allergic asthma, this specific immune response is shifted towards a Th2-type immune response, reflected by a typical cytokine profile, supporting the isotype switch of antibodies towards IgE.

Precursors of antigen-presenting cells in human bone marrow express TrkC receptor [33]. In contrast, human peripheral blood monocytes express TrkA and p75, mediating the survival-promoting effect of NGF [34]. Interestingly, in vitro differentiation of human peripheral blood monocytes into dendritic cells is accompanied by loss of TrkA expression, changing NGF ligation into an apoptotic signal [35]. Thus neurotrophin effects might be involved in the differentiation and maturation processes of antigen-presenting cells, but whether antigen presentation itself is affected by neurotrophins is not yet clear. Opposing data exist concerning an effect of NGF on molecules involved in antigen presentation. One in vitro study showed that TrkA stimulation did not change the expression of MHC, CD86 and other co-stimulatory molecules in human peripheral blood monocytes [35]. A more recent in vitro study instead provides evidence that NGF might have anti-inflammatory effects as it induced down-regulation of MHCIId and CD86 in human monocytes [36]. This seems to be in contrast with the evidence that airway inflammation in asthma is associated with high neurotrophin levels, but it could also be a clue that neurotrophin production might be intended to counter-regulate airway disease and is therefore up-regulated during asthma.

The allergen-induced immune response might be regulated further by neurotrophin effects on lymphocytes. In murine models of asthma, NGF was shown to increase the production of IgE, IgG1 and Th2 cytokines in lymphocytes in vitro, if treated together with allergen [9]. Thus neurotrophins might support IgE production, resulting in allergen-reactive mast cells, reflecting sensitization and further can enhance the Th2-type shifted immune response. Additionally, NGF was described as an autocrine survival factor for memory B-cells [31]. Moreover, a study with BDNF-deficient mice unravelled a pivotal role of BDNF in B-cell development [37], indicating an essential role for the development of an immune response.

Neurotrophins and airway inflammation

As mentioned above, neurotrophins such as NGF can directly stimulate lymphocytes to produce Th2 cytokines, in agreement with the Th2-type shifted immune response and elevated neurotrophin levels during allergic asthma. This is supported by studies in murine asthma models showing that treatment of mice with anti-NGF antibodies decreases Th2 cytokine levels [9,38]. These results indicate a possible role of NGF promoting the typical Th2-type immune response during allergic asthma airway inflammation.

The cells invading into the lung upon mast cell mediator release are susceptible to neurotrophins. Eosinophils from the BALF of patients with allergic asthma show increased expression of neurotrophin receptors after SAP compared with blood eosinophils. Increased expression of neurotrophin receptors in BALF eosinophils was associated with an enhanced survival- and activation-promoting effect of neurotrophins [41]. These results indicate that neurotrophins exert their effects specifically on lung eosinophils. Thus neurotrophin-mediated survival of eosinophils might be involved in the eosinophilia observed after allergen provocation.

Neurotrophins and EAR

The EAR, occurring upon allergen inhalation within minutes, is caused by mast cell mediator release and characterized by acute broncho-obstruction due to smooth-muscle constriction, enhanced mucus secretion and vasodilatation. EAR is at least partly mediated by NGF. Local treatment with anti-NGF antibodies inhibited broncho-obstruction in a murine asthma model, whereas pulmonary NGF overexpression increased broncho-obstruction after allergen provocation [38]. Similar effects were observed in studies with guinea-pigs and rats [42,43]. The enhancement of acute airway obstruction by NGF might be due to its mast cell stimulatory function, indicated by the observed elevated serotonin levels in BALF of NGF-overexpressing mice [38]. NGF can stimulate mast cell degranulation and histamine release via the TrkA pathway in rat peritoneal mast cells [39,40], which might also take place in the lung.

Neurotrophins and AHR

The increased sensitivity of asthmatic patients to sensory stimuli such as cold air or cigarette smoke suggests an involvement of sensory nerve function in the pathogenesis of the disease. Previous results show that NGF treatment of
Figure 1 | Proposed mechanisms of neurotrophins in the airways during allergic asthma

Neurotrophins are produced by different cells in the lung: Ep, airway epithelium; N, sensory neurons; SM, smooth muscle; F, fibroblasts; M, mast cells; Ly, lymphocytes; Eo, eosinophils; MΦ, macrophages. Neurotrophins exert their function on different cells in the airways. (1) Neurotrophins can stimulate mast cell degranulation and augment the production of Th2-type cytokines and IgE antibodies directly by affecting lymphocytes. Moreover, neurotrophins act as survival factors for eosinophils, and therefore augment airway inflammation during allergic asthma. (2) Neurotrophins induce increased sensory nerve neuropeptide content and hyperinnervation as well as up-regulation of neuropeptide receptors in the lung. (3) Neurotrophins activate fibroblasts to differentiate into myofibroblasts, resulting in smooth-muscle hypertrophy and collagen deposition. Additionally, neurotrophins stimulate angiogenesis, contributing to airway remodelling.

mice results in AHR similar to allergen-sensitized and challenged mice [44]. Thus AHR can be provoked by neurotrophins even without the background of allergic airway inflammation. To assess the role of neurotrophins with respect to AHR, neurotrophin receptor knockout mice were used in experimental asthma models. Mice lacking p75NTR showed an almost completely abolished hyperresponsiveness to capsaicin after allergen sensitization, clearly showing a pivotal role of neurotrophins in causing neuronal hyperreactivity [45].

BDNF was shown to contribute to the development of hyperreactive airways, as mice treated with anti-BDNF antibodies even without allergic bronchial hyperreactivity in response to capsaicin [46]. The neuronal hyperreactivity in the lung is possibly caused by functional changes of the sensory nerves induced by neurotrophins. NGF treatment of guinea-pigs resulted in enhanced AHR upon allergen sensitization, accompanied by increased sensory nerve neuropeptide production and release [47]. Additionally, mice overexpressing NGF show enhanced hyperreactivity of sensory neurons to capsaicin [38] and hyperinnervation of substance P-containing sensory nerves [48]. Thus tachykinins such as substance P that are released from sensory nerves may mediate neurotrophin-induced hyperreactivity, as blocking the substance P receptor NK-1 can diminish NGF-induced AHR in guinea-pigs [49]. One mechanism for the neurotrophin-induced increase in tachykinin function might be the up-regulation of NK-1 receptor in lung tissue by NGF [50].

**Neurotrophins and airway remodelling**

Airway remodelling, a feature of chronic airway inflammation [51], is characterized by thickening of the basement membrane, fibrosis, goblet cell hyperplasia, smooth-muscle hypertrophy, increased vascularization and sensory nerve hyperinnervation. Recent results indicate that NGF may be involved in airway remodelling. Beside its effects on bronchial smooth muscle and hyperinnervation with sensory nerves, NGF induces fibroblast migration and differentiation into myofibroblasts, as well as collagen production [52,53]. Additionally, NGF plays a role in increased vascularization by inducing endothelial cell and vascular smooth-muscle cell proliferation and stimulating the release of pro-angiogenic factors [54].

**Conclusions**

Neurotrophins can be produced by various cell types, including structural cells and immune cells in the airways. Most of these cells also express neurotrophin receptors,
indicating an intimate and very complex communication between these cell types by neurotrophins, acting in concert with cytokines and neuropeptides. A survey of cellular sources and target cells of neurotrophins in the lung, as well as their proposed effects during allergic asthma, is provided in Figure 1.

Neurotrophins affect all features of allergic asthma, namely airway inflammation, AHR, neuronal plasticity as well as airway wall remodelling, and are therefore pivotal molecules in asthma pathogenesis. Allergen-induced mast cell degranulation, resulting in acute bronchoconstriction during allergic asthma, might be influenced by neurotrophins, but there is no in vivo evidence so far.

The subsequent airway inflammation, initiated mainly by mast cell mediators, results in massive infiltration of the lung with immune cells producing neurotrophins and therefore amplifying neurotrophin levels. The resulting ‘pathological’ amounts of neurotrophins affect multiple cells. They promote the production of Th2-type cytokines and IgE antibodies and prolong eosinophil and mast cell survival. Neurotrophins also contribute to airway remodelling by stimulating fibroblast proliferation and collagen production, as well as angiogenesis. Additionally, neurotrophins initiate airway hyperreactivity, probably by inducing sensory nerve hyperinnervation, enhanced neuropeptide production and up-regulation of neuropeptide receptors in lung tissue. This might be responsible for late-phase reactions and irreversible changes such as airway remodelling and changed activity of sensory neurons.

Thus interfering with neurotrophin synthesis or receptor ligation locally in the lung is a promising therapeutic strategy for limiting the pathogenesis of asthma.

References

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