Introduction
Nerve growth factor:
Facts in relation to its possible role in asthma
Why study nerve growth factor in relationship to asthma?

In November 1996 a review article appeared carrying the title "Nerve growth factor: from neurotrophin to neurokine" (1). The review described a new role for nerve growth factor (NGF) in inflammatory responses. Hence, the classification of NGF as neurotrophin, a protein that induces outgrowth of neurons, was suggested to be changed into neurokine, indicating a similarity to cytokines and thus a role in immunological responses. This publication by Nobel Prize winner Rita Levi-Montalcini and colleagues was in view with our ideas on the possible role of NGF in allergic asthma. This introduction elaborates on the possible role of NGF in allergic asthma.

Allergic asthma

A reversible airway obstruction, airway inflammation and an increased sensitivity to bronchoconstrictive stimuli characterize allergic asthma, the latter phenomenon is also referred to as airway hyperresponsiveness (2-4). Two distinct phases can be discriminated: an early and a late asthmatic response. The early phase involves acute bronchoconstriction, extensive vascular leakage and mucus hypersecretion, immediately after allergen challenge; this usually resolves within 1-2 hrs. The early asthmatic response is mainly caused by products released from mast cells, as allergen specific IgE, induced during a preceding sensitization period, induces massive mast cell activation at the time of allergen challenge (5). One of the important mediators, released by mast cells and responsible for early phase effects is histamine (6). The early phase can be followed by a late phase several hours after the allergen challenge. Infiltration of inflammatory cells in the airways, epithelial shedding, mucus hypersecretion and bronchoconstriction characterize this phase (7, 8). The late response and airway hyperresponsiveness are associated with increases in airway eosinophils and release of inflammatory mediators and cytokines (4, 7, 9-11). The mechanism by which the allergic response relates to airway hyperresponsiveness and respiratory smooth muscle reactivity is still a matter of debate (recently discussed in 12).

Neurogenic inflammation

Neurogenic inflammation contributes to pathological phenomena in several diseases. For example, in asthma (13), in inflamed skin (14) and in neuropathic pain (15). Neurogenic inflammation involves a change in function of sensory neurons due to inflammatory mediators, thereby inducing an enhanced release of peptides from the nerves (13). Sensory neurons are characterized by their expression of certain peptides, the tachykinins. The pungent derived from red peppers, capsaicin, induces release of tachykinins. Sensory neurons are sensitive to capsaicin, indicating that these neurons are expressing the vanilloid receptor 1 (VR1; 16, 17). Sensory nerves containing tachykinins are also referred to as
excitatory non-adrenergic, non-cholinergic (eNANC) nerves. This as opposed to inhibitory NANC (iNANC) nerves, containing vasoactive intestinal peptide and nitric oxide (48).

Several studies have shown a role for neurogenic inflammation in the induction of airway hyperresponsiveness in animal models, in particular for the tachykinins substance P and neurokinin A. Examples are: 13-hydroxyoctadecadienoic acid-(20), IL-5- (21), ozone- (22), citric acid- (23), and toluene diisocyanate-induced (24) airway hyperresponsiveness. Guinea pigs sensitized to and challenged with ovalbumin, as a model for allergic asthma, show an increase in substance P immunoreactive neurons in nodose ganglia (25, 26). Tachykinin levels are elevated in plasma during the exacerbations of asthma and in lavage fluids after allergen challenge (27, 28). Moreover, protective effects on the induction of allergic airway pathology by neurokinin receptor antagonists have been reported (29).

NGF is able to augment neurogenic inflammation (30) and perhaps in this way plays a role in allergic asthma. NGF specifically upregulates synthesis of products of the preprotachykinin gene, which codes for several tachykinins, such as substance P and neurokinin A (31-36). Moreover, NGF changes the properties of sensory nerve endings by inducing a very fast accumulation of second messengers (37) or phosphorylation of key transduction-related proteins or ion channels, thereby sensitizing the peripheral sensory nerve ending (37, 38). Similarly, over-expression of NGF specifically in the airways of mice leads to an enhanced sensory and sympathetic innervation of the airways. These mice were more sensitive to capsaicin, which induced increases in airway resistance (39).

The protein NGF and its receptors
NGF belongs to the family of neurotrophins, which control the survival, differentiation and maintenance of neurons in the peripheral and central nervous systems (40). NGF is a homodimeric molecule (41). Two molecular forms of NGF exist: 7S NGF and 2.5S NGF molecules (42, 43).

Nerve growth factor can interact with two receptors: either the tyrosine kinase receptor A (trkA) or p75. TrkA is a receptor with tyrosine kinase activity that forms a high-affinity binding site for NGF (Kd = 10^{-11}M, 44). NGF binds to the trkA receptor and the NGF-trkA complex is internalized and retrogradely transported to the nucleus, where mRNA levels for preprotachykinin, the precursor for tachykinins, are affected (34, 35). Alternatively, trkA activation leads, in a tyrosine kinase-dependent manner, to phosphorylation of proteins at the nerve terminal, which can induce changes in the properties of the nerve ending (37, 38). The receptor p75 can bind several neurotrophic factors with nanomolar affinity: NGF, brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and NT-4 (reviewed in 61). The p75 low affinity NGF receptor also causes an upregulation in tachykinin content in sensory nerves (45).
NGF and inflammation
Several inflammatory mediators, including interleukin-1, interleukin-4, interleukin-5, tumor necrosis factor α and interferon-γ, induce release of NGF (46, 47). In addition to neurons, also non-neuronal cells such as mast cells (48), fibroblasts (46), T-cells (49, 50), B-cells (51), eosinophils (52) lymphocytes (51) and airway epithelial cells (53) can synthesize NGF (see figure).

Figure
Besides neurons, different types of inflammatory cells, such as macrophages, mast cells, eosinophils, B-cells and T-cells produce and release NGF. Furthermore, airway epithelial cells produce and release NGF. A number of these cells express the high-affinity receptor for NGF, trkA; e.g. sensory neurons containing substance P (SP), T-cells, B-cells, macrophages and mast cells.

Many inflammatory cells express a high affinity NGF receptor: monocytes (30, 38, 54), mast cells (55), basophils (56), macrophages (57), T-cells (50, 58) and B-cells (59, 60). NGF shows various effects in inflammatory models. This could be relevant in relation to allergic asthma (reviewed in 46). Indeed, NGF promotes inflammatory mediator release from basophils (56), mast cells (54, 62), T- and B-
cells (1, 50, 63), eosinophils (52) and macrophages (64). Furthermore, NGF induces antibody synthesis and secretion from B cells (63), induces differentiation of monocytes into macrophages (65) and is an autocrine survival factor that rescues macrophages (57). NGF has several effects on mast cells: it attracts mast cells (66), induces phenotypic switching of the mast cells (67) and changes the expression of cytokines (68).

**Role of NGF in inflammatory pain**

The role of NGF has been studied extensively in relation to inflammatory pain (69). A local rise in NGF is found in inflammatory pain (69, 70). Furthermore, hyperalgesia can be induced by simply applying NGF locally (71, 72). In inflammatory models, decreasing the amount of available NGF with the use of antibodies reduces inflammatory pain (70, 73-76). Most pain studies suggest that NGF induces pain by an increased release of substance P (15, 77, 78). Furthermore, the number of trkA immunoreactive neurons is enhanced in a model for inflammatory chronic pain (79). Only a few studies suggest a role for NGF in inflammatory pain by affecting immune cell function (73), involving changes in mast cell function (69, 70).

We postulate that similar mechanisms involving NGF in inflammatory pain could play a role in the inflammation in the airways in allergic asthma. The tachykinin substance P induces constriction of the smooth muscle in the airways (6, 80, 81), increase of vascular permeability (14, 80, 82) and have an effect on immune cell function, such as activating and changing the function of mast cells (83-85).

**Hypothesis on a role for NGF in the asthmatic disease**

We postulate a role for NGF in the induction of allergic asthma. We hypothesize that NGF can affect airway function by changing the properties of the sensory nerves in the airways. In order to reveal the role of NGF in asthma, we first studied the effect of NGF on airway function (chapter 2). Thereafter we performed a more mechanistic approach to analyze whether sensory nerve endings are involved in effects of NGF on airway function (chapter 3). Furthermore, we studied the role of NGF in an allergic model of asthma. In chapter 4 the influence of NGF on acute bronchoconstriction induced by allergen challenge is documented. In chapter 5 this is followed by a study on the influence of NGF on airway hyperresponsiveness, inflammation and increase in substance P containing nerves 24 hrs after allergen challenge. In chapter 6 we speculate on the presumed communication between mast cells and sensory neurons in this respect. In the discussion (chapter 7) we summarize and speculate on mechanisms by which NGF could play a role in the asthmatic disease.
A possible role for NGF in asthma

References

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