

Nerve growth factor and asthma

An increasing body of evidence shows that nerve growth factor (NGF) exerts biological activity not only on the central and peripheral nervous system, but also on the immune system thereby influencing allergic diseases and asthma.

(1) NGF circulating levels are increased in patients with allergic diseases and asthma, and are related to the severity of the inflammatory process and disease. In vernal keratoconjunctivitis, NGF plasma levels correlate with the number of mast cells infiltrating the conjunctiva, and NGF mRNA is increased in nasal mucosal scrapings of patients with allergic rhinitis who have high levels of NGF in serum and nasal fluids; NGF is further increased in nasal fluids after specific allergen challenge.

(2) NGF is produced and released by several modulatory and effector cells of allergic inflammation and asthma, for example T-helper 2 lymphocytes, mast cells and eosinophils.

(3) NGF receptors are expressed on the conjunctival epithelium of patients with allergic conjunctivitis and the number of NGF-receptor positive cells is increased in the conjunctiva of these patients. Indeed, local administration of NGF induces fibroblast activation and healing processes of human corneal ulcers, which suggests that NGF plays a role in tissue remodelling processes occurring in asthma.

(4) NGF increases airway hyperreactivity to histamine in an animal model of asthma, while anti-NGF treatment reduces airway hyperreactivity induced by ovalbumin topical challenge in the sensitized mouse.

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Introduction

Koch's postulates have often been adapted to verify whether or not a new molecule is involved in asthma (1). To meet these postulates, the substance should:

1 be found in biological fluids of asthmatic patients in physiological concentrations and be correlated with disease activity;

2 be produced and released by inflammatory cells implicated in asthma;

3 reproduce the key features of the disease;

4 be antagonized by specific treatment with subsequent improvement of asthma symptoms.

The aim of this paper was to review evidence that nerve growth factor (NGF), the best characterized member of the neurotrophin family, meets these postulates and might therefore play a role in inflammatory and remodelling processes of bronchial asthma.

NGF is increased in biological fluids of allergic and asthmatic patients

In 1995 we reported the first observation of increased NGF plasma concentrations in patients affected by vernal keratoconjunctivitis, which is a severe ocular allergic inflammatory disease characterized by mast cells, eosinophils and Th2 lymphocytes (2). Indeed, in

these patients the increased NGF plasma levels were correlated with the increased number of mast cells infiltrating the conjunctiva and with the circulating levels of substance P (3).

One year later we demonstrated that NGF is increased also in the serum of patients with allergic diseases and asthma (4). Nerve growth factor serum levels were measured in 49 patients with asthma and/or rhinoconjunctivitis and/or urticaria angioedema and analyzed in relation to clinical parameters: bronchial reactivity, total and specific serum IgE and circulating levels of eosinophil cationic protein (ECP). Nerve growth factor was significantly increased in the allergic (skin test/RAST-positive) subjects compared with 18 matched controls (49.7 ± 28.8 pg/ml vs. 3.8 ± 1.7 pg/ml). Asthma patients had higher NGF serum values (87.6 ± 59.8 pg/ml) than rhinoconjunctivitis and urticaria-angioedema patients. The increase was more pronounced in asthma patients with sensitization to airborne allergens (132.1 ± 90.8 pg/ml). Serum levels of NGF were correlated with total IgE serum values ($p=0.43$; $p<0.02$), the highest values being found in asthma subjects with a high degree of bronchial reactivity to histamine and high serum levels of ECP.

Subsequently, Braun et al. (5) reported increased NGF concentrations in bronchial alveolar lavage

during late asthmatic responses to allergen challenge, associated with an increased number of eosinophils and Th2 cytokines. In line with this data, Samico et al. demonstrated overexpression of NGF (and NGF-mRNA) in nasal mucosal scrapings of allergic rhinitis patients and increased NGF concentrations in nasal fluids, particularly after allergen challenge (6, 7).

Taken together, these data demonstrate that NGF is increased in biological fluids of patients with asthma and allergic disease and that its levels correlate with disease activity.

NGF is produced and released by inflammatory cells implicated in asthma

In 1994 Leon et al. reported the first observation that rat mast cells synthesize, store and release NGF (8). More recently it has been demonstrated that human mast cells not only produce NGF, but also specifically release NGF after activation through cross-linkage of the high affinity IgE receptor (9, 10).

CD4⁺ lymphocytes with a Th-2-type profile are known to play an important role in modulating allergic inflammatory mechanisms in asthma. Our group has investigated the expression of NGF and NGF receptors by human Th0, Th1 and Th2 cell clones (11). In basal conditions, both Th1 and Th2 clones, but not Th0 clones, secrete NGF in culture supernatants, while phytohaemagglutinin activation induces NGF secretion by Th0 clones and selectively increases NGF release by Th2 clones.

NGF is also produced and released by human circulating eosinophils. In fact, NGF was found in sonicates of blood eosinophils isolated and purified from 7 subjects with mild eosinophilia in concentrations ranging from 1.5 to 17.8 pg/ml/10⁶ cells (12). The production of NGF by eosinophils was confirmed by the demonstration of NGF mRNA in freshly isolated cells.

NGF reproduces the key features of asthma

NGF exerts its biological effects by interacting with two specific receptors: a tyrosine kinase high affinity receptor (TrkA) and a low affinity receptor (p75), which belong to the TNF receptor superfamily (13). NGF receptors are expressed on such allergic inflammatory cells as mast cells (9), CD4⁺ lymphocytes (11) and eosinophils (12). NGF has a priming effect on anti-IgE histamine release from circulating human basophils (14) and induces a selective release of cytotoxic mediators from peripheral blood eosinophils (12).

Mast cells, CD4⁺ lymphocytes and eosinophils also

produce NGF. Therefore, one may envisage that activation and survival of these cells occur via an autocrine/paracrine circuit, which is consistent with the well known effect exerted by NGF on neuronal cells and B cells (13).

In humans, topical administration of NGF causes intense fibroblast proliferation and tissue healing in patients with corneal ulcers (15–17). In animals, the systemic administration of NGF increases, in a dose-dependent fashion, the histamine-induced bronchoconstriction by as much as 200% (18). *In vitro*, NGF displays stimulatory effects on human skin and lung fibroblasts (19). These observations strongly support the hypothesis that NGF plays a role in inflammatory and remodelling processes in allergic disease and asthma.

Anti-NGF treatment reduces airway hyperreactivity

Braun et al. (20) demonstrated that the nasal application of anti-NGF to ovalbumin-sensitized mice significantly reduces IL-4 concentrations and prevents the development of airway hyperreactivity induced by specific allergen challenge. This preliminary observation on the effect of anti-NGF treatment in antagonizing airway hyperreactivity will hopefully prompt further studies on the possibility of modulating allergic inflammation and asthma by reducing the synthesis of NGF or by blocking its activity at specific receptor level.

Concluding remarks

In 1990, Levi-Montalcini et al. (21) were the first to suggest that the biological effects of NGF are not confined to the nervous system but extend to the endocrine and immune systems. In this paper, we have focused on the possible role of NGF in allergic inflammation and asthma. In fact, NGF is produced by and can act on all the major cells involved in allergic inflammatory events that are responsible for symptoms in allergic patients. The marked effects of this molecule on peripheral nerves and the autocrine circuits demonstrated for several inflammatory cells suggest that NGF exerts a modulatory effect on the complex network of inflammatory cells and mediators operating in allergic inflammation and asthma. Indeed, the data obtained *in vivo* about the potent effect of NGF on fibroblast proliferation, collagen deposition and tissue changes during reparatory processes subsequent to inflammatory events suggest that NGF is involved in the remodelling processes that occur in asthma.

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