Perspectives in asthma

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Neurotrophins and asthma: Novel insight into neuroimmune interaction

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There is increasing evidence that neuronal dysfunction and dysregulation contribute to the pathogenesis of allergic asthma. Many functional aspects of peripheral neurons strongly depend on the activity of neurotrophins, a family of mediators originally defined by their neuronal growth activity. More recently, it has been discovered that neurotrophins (eg, nerve growth factor, brain-derived neurotrophin factor, and neurotrophin 3) have profound activities on various immune cells involved in the pathogenesis of allergic disease. Furthermore, immune cells themselves can produce neurotrophins under certain conditions, and the levels of neurotrophins, as well as neurotrophic activities, are strongly upregulated in allergic conditions. Animal data demonstrate that a number of pathomechanisms controlling allergic diseases are directly related to neurotrophin function, including the development of airway hyperresponsiveness. These findings now lead to a much better understanding concerning the regulatory loop between immunologic and neurogenic dysregulation. In this review we will provide an overview of how neurotrophins connect the pathobiology of airway inflammation and hyperresponsiveness, which are the hallmarks of allergic asthma. (J Allergy Clin Immunol 2006;117:67-71.)

Key words: *Asthma, allergy, neurotrophins, airway inflammation, airway hyperresponsiveness, neuroimmune interaction*

Abbreviations used

AHR: Airway hyperresponsiveness
BALF: Bronchoalveolar fluid
BDNF: Brain-derived neurotrophic factor
NGF: Nerve growth factor
NT-3: Neurotrophin 3
NT-4/5: Neurotrophin 4/5
Trk: Tropomyosin-related tyrosine kinase

It is now clear that inflammation and airway hyperresponsiveness (AHR) do not develop independently from one another but rather are associated through bidirectional signaling between cells of the immune and nervous systems. Therefore the search for bidirectional signaling molecules between immune cells and neurons has become a novel focus regarding asthma research. Recent studies have also been conducted investigating the effects of interleukins and other classical cytokines of the immune system on neurons and smooth muscle cells. Vice versa, mediators of the nervous system, especially neuropeptides and neurotransmitters, have been studied for their functional activities in inflammatory cells. During the last decade, the neurotrophins, originally defined as neuronal growth factors, have been identified as important mediators of inflammatory signals regarding the bronchial nervous system. The identification of immune cells, either invading into or residing within the lung, as additional sources of neurotrophins during allergic inflammation has further underlined this concept. In this review we will discuss recent findings demonstrating how increased expression of neurotrophins in the inflamed lung modulates AHR, and we will also indicate the function of neurotrophins as cytokines promoting the allergic inflammation itself.

THE NEUROTROPHINS

Neurotrophins are a family of homologous proteins that have similarities in receptor utility and physiologic activities. The neurotrophins were originally identified as essential factors for the development of the vertebrate nervous system.[1] They regulate the survival, death, or differentiation of neurons in the embryonic and postnatal stages, as well as neuronal maintenance later in life.

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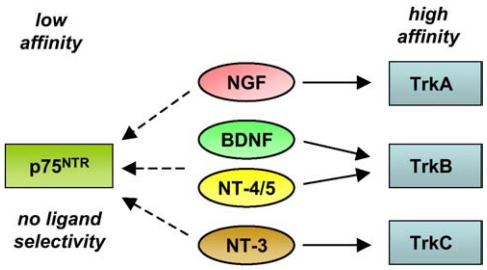

FIG 1. Interaction of neurotrophins and their receptors.

Neurotrophins act as target-derived, paracrine and autocrine growth factors, and all members of this protein family exhibit partial overlap but also distinct patterns of expression and biologic targets. In mammals the neurotrophin family consists of at least 4 members termed nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5). However, additional factors termed neurotrophin 6 and neurotrophin 7 have been identified in other species. All members of the neurotrophin family use a common group of high-affinity cell-surface receptors: the tropomyosin-related tyrosine kinase (Trk) receptors.[2] Additionally, all neurotrophins signal through the common low-affinity neurotrophin receptor $p75^{NTR}$ with faster binding kinetics. The $p75^{NTR}$ is a member of the TNF receptor/Fas/CD40 superfamily and binds all mature neurotrophins with low affinity (Fig 1). In contrast, the Trk receptors bind neurotrophins more specifically, with NGF interacting preferentially with TrkA receptors and BDNF binding preferentially to TrkB. NT-3 interacts with TrkC receptors, and NT-4/5 uses the TrkB receptor together with BDNF. Neurotrophin receptors are widely expressed in neurons of the central and peripheral nervous system, but Trk receptors, as well as $p75^{NTR}$, are also found on nonneuronal cells, including immune cells, smooth muscle and epithelial cells, and fibroblasts.[3]

NEUROTROPHINS IN THE ALLERGIC LUNG

In the central nervous system the primary sources of neurotrophins are nerve-associated cells, such as Schwann cells, glial cells, or fibroblasts, as well as neurons themselves.[1] In the normal lung constitutive expression of neurotrophins is found in the airway epithelia,[4] as well as in alveolar and interstitial macrophages.[5] Under physiologic conditions, the local production of neurotrophins is low because measurements in bronchoalveolar lavage fluid (BALF) revealed only marginal amounts of neurotrophins in both human and animal studies. However, neurotrophin concentrations in BALF dramatically increase during allergic inflammation, both in allergic patients and in mouse models of experimental allergic asthma. Increased concentrations of the neurotrophins NGF, BDNF, and NT-3 were found after allergen inhalation in asthmatic patients,[6,7] and increased levels of NGF[8] and BDNF[9] were observed in allergen-challenged animals.

Potential cellular sources of increased neurotrophin production are resident lung cells but also invading immune cells (Fig 2). The marked induction of neurotrophin production during allergic inflammation implies that cells of the inflammatory response are themselves sources of neurotrophins. Many immune cells, including mast cells, macrophages, eosinophils, and lymphocytes, are able to produce NGF, at least after appropriate stimulation *in vitro*,[10] and immunohistochemical studies have revealed an expression of both BDNF and NGF within the inflammatory infiltrate.[10] However, data showing neurotrophin expression on a single-cell level are still lacking for immune cells within the allergic lung.

There is growing evidence that the respiratory epithelium is the main source of NGF and BDNF, even under inflammatory conditions. The bronchial epithelium is in a key position to translate inflammatory signals between the luminal space and the lung tissue. Airway epithelial cells contribute to the inflammatory reaction through the release of various cytokines, and epithelial neurotrophin expression is upregulated during allergic inflammation,[9] as well as after stimulation with inflammatory cytokines, such as IL-1β and TNF-α.[11] Very recently, we have shown that epithelial-eosinophil interactions trigger enhanced neurotrophin production by airway epithelial cells, which results in prolonged survival of eosinophils (Hahn C et al, article submitted for publication).

Furthermore, some evidence also exists to suggest that fibroblasts and airway smooth muscle cells might be additional sources of NGF during inflammatory activation (Freund and Frossard[12] and our own observation).

NEUROTROPHINS MODULATE AHR

It has been postulated that allergic airway inflammation affects neural activity at several points along the neural reflex pathway, including the function of afferent sensory nerves and synaptic transmission within autonomic ganglia and at the level of the postganglionic neuroeffector junction.[13] After irritation, activation of sensory airway nerves occurs and triggers an axonal response that acts as an immediate protective mucosal defense mechanism, resulting in coughing and sneezing. Coughing, sneezing, and other avoidance mechanisms clear the upper and lower airways from offending agents. There is now growing evidence that changes in airway sensory innervation are under the control of inflammatory mediators released during allergic inflammation,[14] and it is well known that neurotrophin expression is intensively upregulated in the inflamed lung. Functional studies revealed that NGF upregulates the content of neuropeptides in sensory nerves and thus promotes enhanced signal transition after stimulation of these neurons.[15] Thus neurotrophins contribute to AHR through increasing the activity of the peptidergic sensory airway nerves (Fig 2).

Neuropeptides of the tachykinin family (eg, substance P and neurokinins A and B) are the main neurotransmitters of the excitatory sensory nervous system. In animal models

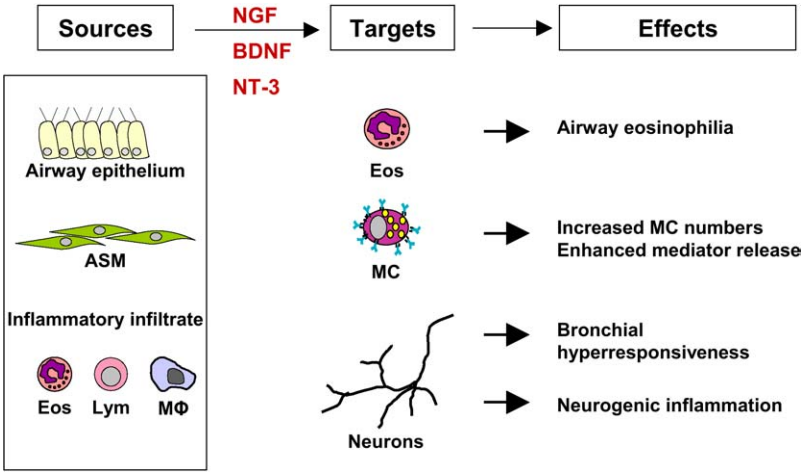

FIG 2. Production and effects of neurotrophins during allergic airway inflammation. *ASM,* Airway smooth muscle cell; *Eos,* eosinophil; *Lym,* lymphocyte; *MΦ,* macrophage; *MC,* mast cell.

of allergic airway inflammation, allergen-challenged animals display an increase in substance P–immunoreactive nodose neurons, and a similar effect was shown after tracheal NGF application.[16] NGF induces a phenotypic switch in sensory airway innervation, such as *de novo* expression of substance P, thus allowing more sensory neurons to become components of the tachykinergic lung innervation.[17] Moreover, NGF also increases the release of stored substance P from sensory nerve endings without the need for neuropeptide synthesis in the neuronal cell body. This has been demonstrated by the observation that NGF can increase airway hyperreactivity in the absence of sensory nerve cell bodies, such as in isolated tracheal rings.[18]

The mechanisms of neurotrophin-mediated AHR have been intensively studied in animal models of allergic asthma by using transgenic mice with alterations in neurotrophin or neurotrophin receptor expression. Sensory airway reactivity is measured through reactivity against capsaicin, which acts specifically through vanilloid receptors expressed on sensory neurons.[19] Hyperreactivity caused by capsaicin-induced irritation of lung sensory neurons is markedly increased in transgenic mice, which constitutively overexpress NGF in the lung epithelium.[20] Vice versa, in transgenic mice lacking $p75^{NTR}$ expression, the capsaicin-induced sensory hyperreactivity is almost completely abolished after allergen sensitization.[21] Determination of tachykinin expression in both transgenic mice strains revealed an enhanced substance P–positive sensory innervation in the lungs of NGF-transgenic mice[22] and a reduced amount of substance P in the lungs of $p75^{NTR-/-}$ mice.[23] On the other hand, alterations in NGF signaling did not alter methacholine-mediated smooth muscle contraction in the same studies.[20,21] However, airway smooth muscle contractility might be indirectly controlled by neurotrophins through alterations of the local axonal reflex circuit. Irritation of sensory neurons is thereby transduced into parasympathetic activity, which directly triggers airway smooth muscle contractility through cholinergic receptors.[24] Thus alterations of sensory innervation by neurotrophins might subsequently also modulate cholinergic bronchoconstrictor pathways. Indeed, an enhanced cholinergic activity has been recently found in explanted mouse tracheal segments after culture with NGF.[25]

NEUROTROPHINS MODULATE AIRWAY INFLAMMATION

In 1996, Bonini et al[26] first described increased blood levels of NGF in patients with allergic asthma, as well as in patients with other allergic inflammatory diseases. Within the lung, the increase in neurotrophin expression parallels the influx of inflammatory cells into the airway tissue, and a direct relationship between neurotrophin production and the local immune response has been postulated. In particular, neurotrophins might influence allergic inflammation at different levels: local recruitment of effector cells (eg, mast cells and eosinophils); maintenance, support, and activation of these cells in the tissue; and direction of the immune response toward a T_H2-specific phenotype with synthesis of IL-4 and IgE. So far, no information is available that neurotrophins directly influence the switch of naive $CD4^+$ T cells into T_H2 cells. However, at least in a transgenic mouse model, NT-3 selectively supports the activity of antigen-specific T_H2 cells but not of T_H1 cells because of an expression of the TrkC receptor on T_H2 but not T_H1 cells.[27]

Many more data underline the effects of neurotrophins on mast cells and eosinophils, representing the main effector cells during the local allergic immune response in the airways. Especially the local accumulation of eosinophils, which are at least quantitatively among the most important cell type within the inflammatory infiltrate, is influenced by neurotrophin signaling. It has been recently shown that eosinophils obtained from the BALF of allergic patients express neurotrophins and their receptors, which promote survival of eosinophils *in vitro*.[28] On the

other hand, peripheral blood eosinophils obtained from the same patients did not express neurotrophin receptors, and they are not susceptible to the survival effects of neurotrophins. Within the tissue, activation of eosinophils followed by induction of neurotrophin receptor expression induces their ability to participate in antiapoptotic neurotrophin signaling, resulting in prolonged survival in the lung. Paracrine delivery of neurotrophins is facilitated by resident cells of the lung (eg, epithelia or fibroblasts), which are strong producers of neurotrophins in inflammatory conditions. Thus enhanced local neurotrophin production supports tissue eosinophilia during allergic inflammation (Fig 2). Consequently, in NGF-transgenic mice, which are characterized by upregulated NGF expression in the airway epithelia, eosinophilic infiltration is markedly enhanced in allergen-sensitized compared with wild-type mice.[20] However, invading immune cells are also sources of NGF themselves. Eosinophils produce and release NGF after stimulation with immune complexes,[29] and thus activated eosinophils might also respond to NGF in an autocrine signaling loop during allergic inflammation.

In addition to eosinophils, the survival of mast cells is, at least partially, regulated by NGF. Mast cells are critical effector cells in allergic tissues and represent key points for immediate hypersensitivity reactions after activation through IgE and specific allergens. Tissue mast cell numbers are dependent on factors controlling infiltration, local development, and survival in the tissues. An increase in mast cell numbers is a characteristic feature of the allergic airways,[7] and several studies suggest that NGF is mainly involved in the development and maintenance of mast cell hyperplasia in the allergic airways (Fig 2). NGF functions as a chemoattractant for mast cells[30] and also supports the survival of mast cells as a cofactor together with stem cell factor.[31] Moreover, NGF stimulates the activity of resident tissue mast cells, such as the release of serotonin,[32] and recent findings from our laboratory revealed increased serotonin release after local allergen challenge of transgenic mice with constitutive overexpression of NGF in the lung compared with that seen in wild-type animals.[20]

NEUROGENIC INFLAMMATION

During allergic inflammation, sensory airway nerves amplify the immune response through the release of neuropeptides and neurotransmitters. This neuronally mediated immune regulation has been previously termed neurogenic inflammation.[33] Thus neurogenic inflammation describes a vicious cycle of neuroimmune interactions that amplify airway inflammation and AHR in allergic asthma. The activities of substance P, the most important neuropeptide released by sensory nerves, include a broad range of functional responses of immune cells, including lymphocytes, eosinophils, mast cells, and macrophages, leading to activation and differentiation of these cells.[34,35]

Although immunoregulation of neuropeptides has been intensively studied, one important question is related to the mechanism of neurotransmitter induction during the inflammatory process. Various interleukins (eg, IL-1) mediate signals from the immune to the nervous system and activate tachykinin synthesis[35]; however, communication between neurons and immune cells is not restricted to these classical cytokines. The neurotrophins are now recognized to contribute to the main mediators of neurogenic inflammation through their biologic activities in both the nervous and immune systems (Fig 2).

It is well known that neurotrophins modulate the activity of sensory neurons and enhance the synthesis and release of neuropeptides.[36] Local overproduction of neurotrophins during allergic inflammation results in increased neuronal release of substance P after stimulation, and immune cells contribute to this process by virtue of their neurotrophin expression. Therefore neurotrophins can influence the intensity and duration of a local immune response either by direct signaling through specific neurotrophin receptors or through regulation of neuropeptide synthesis in the local nervous system. Because neuropeptides are short-lived signaling molecules that are rapidly degraded,[37] their action is temporally limited and mainly restricted to the site of synthesis. Neurotrophins, however, were found to be produced continuously during allergic inflammation, and their concentrations in the BALF increase during repeated allergen provocation in mice (Hahn C et al, article submitted for publication). Thus they might act as long-term modulators, amplifying inflammatory signals between the nervous and immune systems during neurogenic inflammation.

CONCLUSION

In allergic asthma the development of allergic inflammation and neuronal hyperresponsiveness are functionally associated. One group of mediators of this neuroimmune interaction is the neurotrophin family, members of which are intensively produced in the allergic lung. Originally described as exclusive neuronal growth and survival factors, many functional effects have been identified that lie beyond the nervous system. It is now being recognized that enhanced local neurotrophin expression modulates activity of the peripheral nervous system, and alterations in sensory innervation are an important feature in the pathogenesis of allergic asthma. On the other hand, more recent data indicate that neurotrophins also promote the development and degree of the allergic immune response. Neurotrophins are produced by epithelia, fibroblasts, and immune cells, all of which contribute to the control of local inflammation. Thus neurotrophin expression and signaling in the allergic airways can contribute to the complex neuroimmune interactions in the pathophysiology of allergic asthma and might become a potential therapeutic target in the future.

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