Allergy and the Nervous System

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Neurotrophins in Chronic Allergic Airway Inflammation and Remodeling

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Abstract

Allergic asthma is a chronic inflammatory disease characterized by the production of allergen-specific IgE antibodies, TH2 inflammation, airway hyperresponsiveness and airway remodeling. Airway remodeling represents the disease-limiting stage during disease progression, and the underlying cellular molecular network resulting in airway remodeling are still poorly defined. In addition to the well-established TH2-dependent inflammatory response, several lines of investigation reveal that this regulation in the peripheral central nervous system contributes to disease development, exacerbation and progression. Several members of the neurotrophin family (e.g. nerve growth factor, brain-derived neurotrophic factor) are important transmitters of signals between the immune and the nervous system. Recent data indicate that NGF contributes to the development of airway remodeling in an inflammation and TGF-independent manner. These and other data open the opportunity to therapeutically interfere also on this level of regulation as a novel approach.

Neurotrophins (NTs) comprise a family of homologous proteins, showing similarities in receptor utilization and activity. Initially identified for their critical role in promoting neuronal growth, survival and differentiation [1], there is growing evidence for an eminent role of NTs exceeding the nervous system. NTs, which are elevated systemically and locally in the lung during allergic asthma, serve as ‘trophic’ factors for different immune cells, including cells of the innate and adaptive immune system. They promote survival, proliferation and differentiation as well as mediator release by immune cells. Beyond this immunomodulatory role of NTs, a direct role in controlling structural cell activity during inflammation and repair process has been established. These findings implicate a critical role for NTs in deregulated and chronic inflammatory processes, which finally lead to tissue remodeling in allergic asthma. This chapter summarizes the knowledge of NTs in allergic asthma and remodeling processes in the chronic inflamed lung.
Neurotrophins: Family Members and Synthesis

Classically, the NT family comprises four proteins, all similar in structure and function: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3 and NT4/NT5.

NTs are synthesized as preproprotein precursors of ~27 kDa, which are cleaved intracellularly by furin or pro-hormone convertases to form pro-NTs [2]. These pro-NTs are either packed into constitutive vesicles in the case of pro-NGF, pro-NT3 and pro-NT4/NT5, or pro-BDNF is loaded into regulated secretory vesicles for secretion. Extracellularly, these pro-NTs are further processed by plasmin or the matrix metalloproteinases (MMP) 3 and 7 to release the mature NTs with a molecular weight of ~13–15 kDa. Mature NTs BDNF, NT3 and NT4 share approximately 50% sequence identity with NGF and all show a high degree of homology across species (90–100%).

Neurotrophin Receptors and Basics of Neurotrophin Signaling

NTs exert their divergent biological effects by utilizing two structurally unrelated receptor systems: the tropomyosin-related tyrosin kinases (Trk) and the ‘pan-neurotrophin’ receptor of 75 kDa (p75NTR) (Fig. 1).

Trk receptors, with molecular weights of 140–145 kDa, are preferentially activated by the binding of mature NT dimers (dissociation constant \([K_d] 10^{-11} \text{ m}\)) for which they show ligand specificity. The TrkA receptor preferentially binds NGF, while TrkB interacts with BDNF and NT4/NT5 and the TrkC receptor is activated by binding of NT3 [3, 4]. These receptors are expressed in both full-length and truncated isoforms [3]. Although the expression of truncated receptor isoforms is documented, their functional role remains unclear. Biological activity of NTs requires binding to full-length Trk receptors.

Binding of an NT dimer leads to dimerization of two receptor chains and subsequent auto-phosphorylation. Intracellularly, three main pathways are activated: phospholipase C \(\gamma\) (PLC\(\gamma\)), phosphatidylinositol 3-kinase (PI3K) and the mitogen-activated protein kinsases (MAPK), extracellular regulated kinase (ERK) and p38MAPK [5]. Signaling via Trk receptors induces activation, migration and survival in the target cell.

The p75NTR belongs to the tumor necrosis factor (TNF) receptor/Fas/CD40 superfamily. It binds all four NTs, preferably the pro-NTs, with the same affinity \([K_d] 10^{-9} \text{ m}\). Binding of NTs leads to trimerization of the receptor chains and subsequent activation of intracellular pathways, which evoke two different effects in the signal perceiving cells. Activation of c-jun terminal kinase (JNK) and subsequent phosphorylation of pro-apoptotic proteins, including Bad, p53 and Bax, induces cytochrome c release from mitochondria. This further leads to caspase-3 and caspase-9
activation, eventually leading to apoptosis. In contrast, recruitment of TNF-receptor-associated factor-6 (TRAF6) and activation of interleukine (IL)-1 receptor associated kinase (IRAK) in combination with atypical recruitment of receptor interacting protein-2 (RIP-2) by PKC and activation of PI3K and Akt leads to activation of nuclear factor-κB (NF-κB). NFκB induces transcription of anti-apoptotic proteins of the Bcl-2 family and leads to survival of the target cell [6]. Due to alternative splicing of the p75NTR gene, a truncated isoform of the receptor is expressed. This splice variant lacks exon III, which codes for the NT-binding domain. This protein is transcribed and translated, as well as transported to the membrane, but unable to bind NTs and therefore inactive. For p75NTR cleavage of the receptor on both extra- and intracellular level has been described. Cleavage of the extracellular portion near the plasma membrane by alpha-secretases releases a fragment containing the NT-binding domain. This domain can bind soluble NTs, prevent their binding to functional receptor chains and therefore acts as a scavenger receptor. A presenilin-dependent intramembrane

**Fig. 1.** NT receptors. NTs activate receptor tyrosine kinases (Trk) and p75NTR. NGF preferentially binds to TrkA, BDNF and NT4/NT5 activates TrkB and NT3 signals via the TrkC receptor. The p75NTR is binds all NT, preferentially the pro-NT, with the same affinity. Activation of Trk receptors induces activation, differentiation, proliferation and survival of the target cell, while p75NTR activation can induce apoptosis.
proteolysis of p75NTR yields a soluble p75 intracellular fragment. This fragment can activate transcription factors, like NFκB, by recruiting TRAF6 and even translocate into the nucleus and act by itself as a transcription factor [7].

Besides the structural differences of these two receptor systems, the biological effect exerted in the target cell differs as well. Signaling through Trk receptors is always associated with activation, differentiation and survival of the target cell. In contrast, activation of the p75NTR can either induce apoptosis or survival in the responding cell. Apoptosis is induced by the activation of pro-apoptotic JNK, which finally leads to caspase activation and DNA fragmentation, and induction of ceramide synthesis. Therefore, the expression of each or both receptors determines cell fate. Depending on the signaling pathways activated and adaptor proteins recruited in the target cell, TrkA and p75NTR signaling systems can act independently, counteract each other, or p75NTR signaling can have an additive effect on Trk signaling by forming high-affinity binding sites.

**Neurotrophin Expression in the Lung**

The expression of receptors and cognate ligands by different cell populations at different stages helped to define the role of NTs in maintaining lung structure and function. Using human biopsies as well as mouse studies, the functional role of NT signaling in the lung is being elucidated (Fig. 2).

Already in the developing mouse lung, a dynamic expression pattern of increasing versus decreasing NGF versus BDNF levels is documented. Although changes in NT levels were shown in the developing lung, the functional importance of differing NT levels remains elusive. Studies aiming to investigate NT-signalling during development are limited. This is attributable to the fact, that transgenic animals, lacking functional Trk-receptors, display early mortality. This far, one study provides evidence for the importance of NT signaling in the correct development of the lung. In this study, a mutation in the trkB gene, resulting in the expression of a nonfunctional protein, causes a thinner bronchial epithelium, larger luminal airway diameter and larger air spaces, but thickened vascular walls compared with wild-type animals [8]. Given the fact that lack of BDNF expression does not result in such a phenotype [9], these changes seem to rely on the expression of nonfunctional TrkB receptors. Although data investigating NT function during lung development are limited these observations hint towards or role of NT, especially BDNF in homeostasis of the lung tissue.

Studies performed on adult lung biopsies indicate that NTs and their receptors are expressed by a variety of lung resident cells. For airway epithelial cells constitutive expression of NGF, BDNF and NT3 is documented [10–12], while these studies failed to show the expression of NT receptors. Studying injured rodent lungs and activated airway epithelial cells in vitro, the expression of TrkA could be confirmed
on proliferating epithelial cells [13]. In the presence of an inflammatory stimulus, provided by TNF-α and IL-1β [14], an augmented expression of epithelial NT was observed in vitro. These findings indicate that NGF signaling is active in the injured epithelium and silenced in the normal lung.

Beneath the airway epithelium, a thin layer of pulmonary fibroblasts resides. Using human bronchial biopsy sections NT expression by these cells was shown. Although the functions of NT in lung fibroblasts are still being explored, there is evidence for a prominent role of NGF. The expression of NGF as well as its receptors TrkA and p75NTR could be confirmed in cultured human and murine pulmonary fibroblasts [15, 16]. Besides the above-mentioned structural cells, there is evidence for NT production by smooth muscle cells as well. In lung sections of nonasthmatic human subjects, constitutive expression of the ligands NGF, BDNF and NT3 by airway smooth muscle cells (ASM) was detected [12]. While expression of TrkB could be confirmed by immunocytochemistry, p75NTR was not. Yet, more recent studies performed on isolated human bronchial smooth muscle cells, show the expression of BDNF, NT-3 and NT-4 as well as the receptors TrkB, TrkC and p75NTR [17, 18]. In the presence of an inflammatory response, when cytokines like IL-1β and TNF-α are present, ASM strongly upregulate the expression of NT. Using the model of isolated bronchial ASM, IL-1β induced expression of NGF and BDNF, as well as TNF-α increased BDNF and NT-3 levels are documented. In contrast, the proinflammatory cytokine interferon (IFN)-γ selectively increases NGF-levels while decreasing BDNF expression [19,
In contrast to NT function in bronchial ASM, the role of NT in vascular smooth muscle cell biology is less well defined. Thus far, the expression of NGF and p75<sup>NTR</sup> were identified.

**Neurotrophin Expression in the Immune System**

Allergic diseases are characterized by a unique activation pattern of innate and adaptive immune cells resulting in cellular and humoral immune responses such as eosinophilia and production of immunoglobulin (Ig) E antibodies.

Although NTs were discovered and described as neuronal growth factors, the expression of NTs and NT receptors has been reported in various cell types. Especially immune cells are capable of expressing the ligands and several NT receptors depending on the developmental and activation state (Fig. 3).

**Lymphocytes.** Based on cognate and noncognate signals naïve CD4+ T cells perceive during sensitization to an allergen, their differentiation T helper type 2 (TH2) cells occurs. By secreting characteristic soluble mediators, these cells shape and orchestrate the subsequent inflammatory response, inducing IgE production in B cells, maturation of eosinophils in the bone marrow and differentiation of epithelial cells into mucus-producing cells. The expression of NGF and TrkA but not
p75NTR is documented in TH2 cells. In contrast to TH2 cells, in TH1 cells as another inflammatory subset neither the expression ligands nor receptors could be confirmed, linking NT to helper type 2-mediated diseases. Same as TH2 cells, B cells are not only producers [21, 22] but also responders to NT signaling. For B cells, the expression of ligands as well as both NGF receptors, Trks and p75NTR, is documented [23]. Yet the expression pattern of the receptors seems to be restricted to different developmental and differentiation stages. While the expression of BDNF and its receptor TrKB is linked to proper development of B lymphocytes, mature and finally differentiated CD138+ plasma cells rather express NGF and TrkA as well as NT3 and TrkC [21].

Mast cells, as resident tissue cells, are critical effector cells in allergic diseases. Upon binding of allergens to membrane bound IgE receptors, these cells become activated and release a plethora of soluble mediators, directly contributing to immediate hypersensitivity reactions. Mast cells express NGF, BDNF and NT3 as well as their corresponding receptors TrkA, TrkB and TrkC [24]. Besides being a target cell population for NTs, mast cells produce NGF, BDNF and NT3. Upon IgE cross-linking triggered release of NGF occurs [25], suggesting that allergens induce NT release in diseases such as asthma.

Eosinophils represent the major cell population infiltrating allergic inflamed airways. They differentiate from precursors in the bone marrow and, after short systemic circulation, home to the inflamed tissue. Depending on the organ compartment, maturation and activation state, eosinophils are able to express all NT receptors. In bone marrow, the expression of TrkB and TrkC, but not TrkA and p75NTR, is documented [26]. The expression of NT receptors in peripheral blood eosinophils is controversially discussed. While one study was able to show the expression of all receptors on peripheral blood eosinophils showing varying levels of Trk- mRNA and protein levels among patients [27]. Another study was not able to detect these receptors in eosinophils recovered from allergic patients. In contrast, eosinophils recovered from bronchoalveolar lavage (BAL) after allergen provocation of allergic asthmatics express all NT receptors. Besides representing a target cell population for NTs, eosinophils are possible cellular sources for these soluble mediators [28].

Monocytes and macrophages were identified as strong producers of NGF and BDNF in humans and animals. Human peripheral blood monocytes constitutively express BDNF [29] and NGF [30]. In allergic patients, the cellular content as well as the release of NGF was found to be significantly enhanced compared to healthy donors. Whereas BDNF and NT3 levels did not differ among both groups [31]. For alveolar macrophages capable of producing cytokines critically involved in the induction and propagation of an allergic immune response [32], an elevated expression of NGF and BDNF is documented after allergen challenge in a murine model of allergic airway inflammation [33, 34]. Pro-inflammatory cytokines, including IL-6 and TNF-α, which are released after monocytes/macrophage activation, induce the secretion of BDNF by these cells [35].
While these data clearly identify immune cells as major sources for NT production during allergic inflammation, the affected target cell populations, either other immune or resident structural cells, are still under investigation. It is very likely that NTs modulate the expression of cytokines and receptors in immune cells, making these cells more susceptible to the surrounding milieu and therefore shaping or shifting the pattern of inflammation. Evidence for such an effect is reported in dendritic cells (DC). Depending on the atopic state of a subject, NTs exert opposing effects on TrkA and TrkB expressing DCs. Upon NGF and BDNF treatment, dendritic cells from healthy subjects produce anti-inflammatory IL-10, while DCs from allergic patients secrete pro-inflammatory IL-6 [36].

The diverse expression pattern of NT and their receptor systems combined with the versatile functions elicited in different cell populations implicates a key role for NT in complex inflammatory diseases. In chronic inflammatory diseases both immune as well as structural cells are constantly activated and, with the release of a variety of soluble mediators, control the ongoing immune response which finally affects organ function.

**Neurotrophins in Atopic Diseases**

NT levels are low under physiologic conditions; however, their levels increase significantly in allergic diseases. In bronchial asthma, high levels of systemic NGF, BDNF and NT3 [37] as well as enhanced local expression of NGF and BDNF are detectable in BAL fluid [38]. Local expression of these NTs is further augmented by allergen provocation in both human and mouse [38], suggesting that allergen challenge represents a strong trigger for NT production in the airways. In other atopic diseases, including allergic rhinitis and atopic dermatitis, elevated levels of peripheral blood and local, including nasal lavage and skin, NGF and BDNF are reported [39]. In atopic dermatitis, these increased NT levels show a positive correlation with severity of the disease. Although, these data show a clear association between allergic diseases and NTs, the sources of NTs as well as target cell populations and functional consequences in target cells are still being defined.

**Neurotrophins in Chronic Allergic Airway Inflammation and Remodeling**

During the ongoing allergic immune response, infiltrating immune cells secrete a plethora of toxic metabolites, growth factors and cytokines, which induce proliferation of resident structural and neuronal cells resulting in morphological and functional changes of the lung tissue. A consequence of this augmented release of inflammatory mediators is neuronal dysfunction. Yet, there is growing evidence that neurons and activated resident structural cells not only represent target cell