

## Neuroimmune crosstalk in asthma: Dual role of the neurotrophin receptor p75NTR

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**Background:** Neurotrophins have been implicated in the pathogenesis of asthma because of their ability to induce airway inflammation and to promote hyperreactivity of sensory neurons, which reflects an important mechanism in the pathogenesis of airway hyperreactivity. Neurotrophins use a dual-receptor system consisting of Trk-receptor tyrosine kinases and the structurally unrelated p75NTR. Previous studies revealed an important role of p75NTR in the pathogenesis of allergic asthma.

**Objectives:** The aim of the study was to investigate the precise mechanisms of neurotrophins in neuroimmune interaction, which can lead to both airway inflammation and sensory nerve hyperreactivity *in vivo*.

**Methods:** Mice selectively expressing p75NTR in immune cells or nerves, respectively, were generated. After sensitization and allergen provocation, hyperreactivity of sensory nerves was tested in response to capsaicin. Airway inflammation was analyzed on the basis of differential cell counts and cytokine levels in bronchoalveolar lavage fluids.

**Results:** Allergic mice selectively expressing p75NTR in immune cells showed normal inflammation but no sensory nerve hyperreactivity, whereas mice selectively expressing p75NTR in nerve cells had a diminished inflammation and a distinct sensory nerve hyperreactivity.

**Conclusion:** Our data indicate that p75NTR plays a dual role by promoting hyperreactivity of sensory nerves and airway inflammation. Additionally, our study provides experimental evidence that development of sensory nerve hyperreactivity depends on an established airway inflammation in asthma. In contrast, development of airway inflammation seems to be independent from sensory nerve hyperreactivity.

**Clinical implications:** Because of its dual function, antagonization of p75NTR-mediated signals might be a novel approach in asthma therapy. (J Allergy Clin Immunol 2007;119:1016-1024.)

**Key words:** Neuroimmune interaction, airway hyperreactivity, airway inflammation, sensory nerves, neurotrophin receptors, p75NTR, capsaicin, asthma, bone marrow chimeras, neurotrophins

Bidirectional neuroimmune interaction is believed to be involved in several immunologic disorders that are accompanied by acute or chronic inflammation. The basis for this assumption is provided by structural and functional associations of different subsets of nerves and immune cells in several inflammatory diseases,<sup>1</sup> including allergic asthma.<sup>2,3</sup> Allergic asthma is characterized by recurrent and reversible episodes of broncho-obstruction, airway inflammation, and airway hyperreactivity in response to various unspecific stimuli, including smoke, ozone, and cold air. In addition to changes in airway smooth muscle cells, which are reflected by an enhanced bronchoconstriction in response to methacholine, it has been postulated that allergen-induced sensory neuroplasticity in the airways might contribute to airway hyperreactivity.<sup>4</sup> Sensory neuroplasticity is associated with changes in neuronal excitability and characterized by an enhanced synthesis of neuropeptides in nodose primary afferent neurons, which is, at least in part, due to an increasing number of tachykinin-immunoreactive nodose ganglion neurons projecting to the airways.<sup>5</sup> The rapid release of neuropeptides from peripheral nerve endings is thought to result in airway smooth muscle contraction<sup>6</sup> and might also modulate allergic airway inflammation through direct interaction with immune cells (neurogenic inflammation).<sup>7</sup> In experimental studies capsaicin provocation has been used to study the functional effects of sensory neuroplasticity ("sensory nerve hyperreactivity") in the airways<sup>8,9</sup> because its receptor, TrpV1, is selectively expressed in sensory nerves.

Although allergic airway inflammation contributes to changes in the excitability of sensory nerve endings and the induction of neuropeptide production in airway sensory nerves,<sup>5,10</sup> the mechanisms of allergen-induced nerve activation have not been completely elucidated. Mediators released by immune cells recruited to the airways after allergen exposure might be responsible for the functional

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*Abbreviations used*

BALF: Bronchoalveolar lavage fluid  
 BDNF: Brain-derived neurotrophic factor  
 BM: Bone marrow  
 KO: Knockout  
 NGF: Nerve growth factor  
 OVA: Ovalbumin  
 Tb: Time of braking  
 TBS: Tris-buffered saline  
 WT: Wild-type

changes of pulmonary sensory C-fibers in asthma.<sup>11</sup> Growing evidence suggests that neurotrophins, which are elevated in bronchoalveolar lavage fluid (BALF) after allergen provocation,<sup>12,13</sup> are important candidates. These factors are synthesized by different structural and inflammatory cells within the lung.<sup>14</sup> They can sensitize afferent C-fibers to various irritants and are potent regulators of neuropeptide synthesis in sensory neurons.<sup>10,15</sup>

The relevance of the neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3, and neurotrophin 4 in allergic asthma has been demonstrated in animal models, as well as in patients, indicating that these factors contribute to an altered neuronal control of the airways,<sup>16,17</sup> induce allergic airway inflammation by promoting survival and activation of eosinophils,<sup>18</sup> and augment T-cell cytokine synthesis.<sup>12</sup>

A large proportion of the effects of neurotrophins are probably dependent on the low-affinity pan-neurotrophin receptor p75NTR because disruption of p75NTR signaling inhibited the development of a hyperreactivity of sensory C-fibers and diminished airway inflammation.<sup>8</sup> These data were supported by means of immunohistologic analyses, which revealed a distinct immunoreactivity for p75NTR exclusively in nerve fibers surrounding the airways and in immune cells of the inflammatory infiltrate.<sup>8</sup>

Although recent data indicate a critical involvement of neurotrophins in asthma by binding to p75NTR, the precise mechanisms by which neurotrophins lead to both airway inflammation and sensory nerve hyperreactivity *in vivo* are not known. p75NTR-dependent airway inflammation can be induced by interaction of neurotrophins with immune cells or indirectly by neurotrophin-mediated neuropeptide release from sensory nerves. Conversely, hyperreactivity of sensory nerves might depend directly on neurotrophins or on neurotrophin-mediated mediator release in immune cells, including neuropeptides or eosinophil cationic protein.<sup>11</sup>

We therefore investigated the effect of p75NTR-mediated neurotrophin signaling selectively in nerves and immune cells by using a bone marrow (BM) transfer approach. Our results indicate that p75NTR plays a dual role in allergic asthma. This receptor is involved in airway inflammation by mediating neurotrophin signals in immune cells, most likely T lymphocytes, and in the development of sensory nerve hyperreactivity by

mediating neurotrophin signaling in afferent C-fibers. Because of the observation that p75NTR plays a dual role in asthma, the BM transfer experiment also represented an excellent model to provide experimental evidence that the allergen-dependent development of sensory nerve hyperreactivity critically depends on an established airway inflammation. Moreover, we demonstrate that changes in sensory nerve function do not contribute to the development of airway inflammation (neurogenic inflammation) *in vivo*.

## METHODS

### Animals

Ngfr<sup>tm1Jae</sup> (p75NTR knockout [KO]) mice and their wild-type (WT) littermates (C57BL/6J) were purchased from Jackson Laboratory (Bar Harbor, Me). KO mice were generated through targeted mutation of the gene encoding p75NTR.<sup>19</sup> Mice were backcrossed for 12 generations to a C57BL/6J background and maintained in a homozygous fashion. They were fed with ovalbumin (OVA)-free laboratory food and tap water *ad libitum* and held in a regular 12-hour dark/light cycle at a temperature of 22°C. All animal care practices and experimental procedures were performed in accordance with the German animal protection law under a protocol approved by the appropriate governmental authority (Bezirksregierung Hannover).

### Irradiation and BM transfer

T cells from female donor mice (WT or KO mice, respectively) were depleted by anti-Thy 1.2 antibodies (clone 30H12) before cervical dislocation to avoid graft-versus-host disease in recipients. BM was flushed from donor femurs, passed through sterile mesh filters, washed, and resuspended in PBS.

Recipient mice (female, 6–8 weeks of age) obtained a single intravenous injection of  $5 \times 10^6$  BM cells after irradiation (11 Gy) on day –56 followed by antibiotic treatment (Borgal, Intervet, Unterschleißheim, Germany; 120 µg/mL sulfadoxine and 24 µg/mL trimethoprim in drinking water) for 42 days (see Fig E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Genotyping

A detailed protocol for the genotyping by means of real-time PCR is described in the Methods section of the Online Repository at [www.jacionline.org](http://www.jacionline.org). Genotyping was performed in at least 3 animals per transfer group on blood leukocytes on day –7 to confirm complete reconstitution of peripheral blood cells before sensitization in recipient mice. Complete reconstitution of BALF cells was verified by means of genotyping in at least 3 animals per transfer group after dissection on day 29 (see Fig E2 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Sensitization and allergen challenge of mice

Mice of each group were randomized according to their body weights at day –7 and subdivided into 2 subgroups (Table I). The OVA groups were sensitized with 10 µg of OVA/1.5 mg of Al(OH)<sub>3</sub> in PBS, and the PBS groups were treated with PBS/1.5 mg of Al(OH)<sub>3</sub>. All animals received OVA aerosol provocation on days 28 and 29 (see Fig E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Immunohistochemistry and flow cytometry

Detailed protocols are shown in the Online Repository at [www.jacionline.org](http://www.jacionline.org).

**TABLE I.** Chimeric mice used to test the role of p75NTR and the importance of neuroimmune interaction in a murine model of asthma

| Group         | Irradiation | Donor | Recipients | p75NTR expression |                | Sensitization | No. |
|---------------|-------------|-------|------------|-------------------|----------------|---------------|-----|
|               |             |       |            | Immune cells      | Sensory nerves |               |     |
| WT → WT (PBS) | +           | WT    | WT         | +                 | +              | PBS           | 16  |
| WT → WT (OVA) | +           | WT    | WT         | +                 | +              | OVA           | 16  |
| KO → WT (PBS) | +           | KO    | WT         | –                 | +              | PBS           | 11  |
| KO → WT (OVA) | +           | KO    | WT         | –                 | +              | OVA           | 11  |
| WT → KO (PBS) | +           | WT    | KO         | +                 | –              | PBS           | 15  |
| WT → KO (OVA) | +           | WT    | KO         | +                 | –              | OVA           | 15  |
| KO → KO (PBS) | +           | KO    | KO         | –                 | –              | PBS           | 17  |
| KO → KO (OVA) | +           | KO    | KO         | –                 | –              | OVA           | 17  |
| WT (PBS)      | –           | NA    | NA         | +                 | +              | PBS           | 9   |
| WT (OVA)      | –           | NA    | NA         | +                 | +              | OVA           | 9   |
| KO (PBS)      | –           | NA    | NA         | –                 | –              | PBS           | 9   |
| KO (OVA)      | –           | NA    | NA         | –                 | –              | OVA           | 9   |

NA, Not applicable.

### Assessment of lung function

Reactivity of sensory airway nerves was measured on days 1 and 29 in response to capsaicin aerosol by means of head-out body plethysmography in conscious, restrained, and spontaneously breathing animals, as previously described.<sup>8</sup> A detailed protocol is described in the Online Repository at [www.jacionline.org](http://www.jacionline.org).

### BALF

Twenty-four hours after the last allergen challenge, bronchoalveolar lavage and differential cell counts were performed, as previously described.<sup>12</sup> A detailed description is provided in the Online Repository at [www.jacionline.org](http://www.jacionline.org).

### Assessment of cytokine levels in BALF

The measurement of cytokines and neurotrophins in cell-free BALF supernatants was performed according to the manufacturer's recommendation (IL-4, IL-5, IFN- $\gamma$ , and NGF: DuoSet ELISA Development Kit, R&D Systems, Germany; BDNF: BDNF Emax Immunoassay System, Promega, Mannheim, Germany). Detection limits were 31.25 pg/mL for all cytokines and 15 pg/mL for all neurotrophins. All measurements were performed in duplicate.

### Statistical analysis

Statistical analysis was performed with GraphPad Prism Version 4.0 software (GraphPad Software, Inc, San Diego, Calif). Results are presented as means  $\pm$  SEM.

Normality distribution was assessed by using the Kolmogorov-Smirnov test. All data followed a normal distribution. One-way ANOVA combined with the Bonferroni test for selected pairs of columns was performed to detect differences among selected groups. Two-way ANOVA was used to detect differences among groups in their response to capsaicin during lung-function measurement.

## RESULTS

### Generation of BM chimeras

BM cells from donor mice were transferred into lethally irradiated (11Gy) recipient mice (see Fig E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). Control experiments revealed that application of Thy1.2 antibodies led to depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in peripheral blood,

whereas Sca<sup>+</sup> stem cells in the BM were not affected (data not shown). Reconstitution of blood leukocytes (day –7) and BALF cells (day 29) was verified by using LightCycler PCR in at least 3 animals per transfer group. Both melting curves and PCR product lengths revealed complete reconstitution of all WT BM recipients, as well as KO BM recipients (see Fig E2 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Localization of p75NTR expression in murine airways

Cellular distribution of p75NTR in the murine lung was investigated by means of immunohistochemistry (WT [PBS] and WT [OVA] mice) and flow cytometry (WT [OVA] mice) on day 29.

Immunoreactivity for p75NTR was detected in large bundles of nerve fibers, as well as on terminal subepithelial varicosities, as previously described.<sup>17</sup> Many nerve fibers were positive for both p75NTR and Substance P, indicating that p75NTR is expressed in sensory nerves adjacent to the airways (see Fig E3, A-C, in the Online Repository at [www.jacionline.org](http://www.jacionline.org)).

Furthermore, p75NTR was expressed in cells within the inflammatory infiltrate in WT (OVA) mice, although not in parenchymal cells (eg, the epithelium of intrapulmonary airways; see Fig E3, D-I, in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). Quantitative analysis of p75NTR surface expression in different BALF cell subpopulations by means of flow cytometry revealed that this receptor is expressed in CD3<sup>+</sup> lymphocytes. In contrast, p75NTR surface expression was not seen in BALF granulocytes, consisting of more than 80% eosinophils and macrophages, respectively (see Fig E3, J-L, in the Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Expression of p75NTR on sensory nerves is required for the development of sensory nerve hyperreactivity

The capsaicin-induced time of braking (Tb) after allergen challenge was analyzed in all groups to delineate the

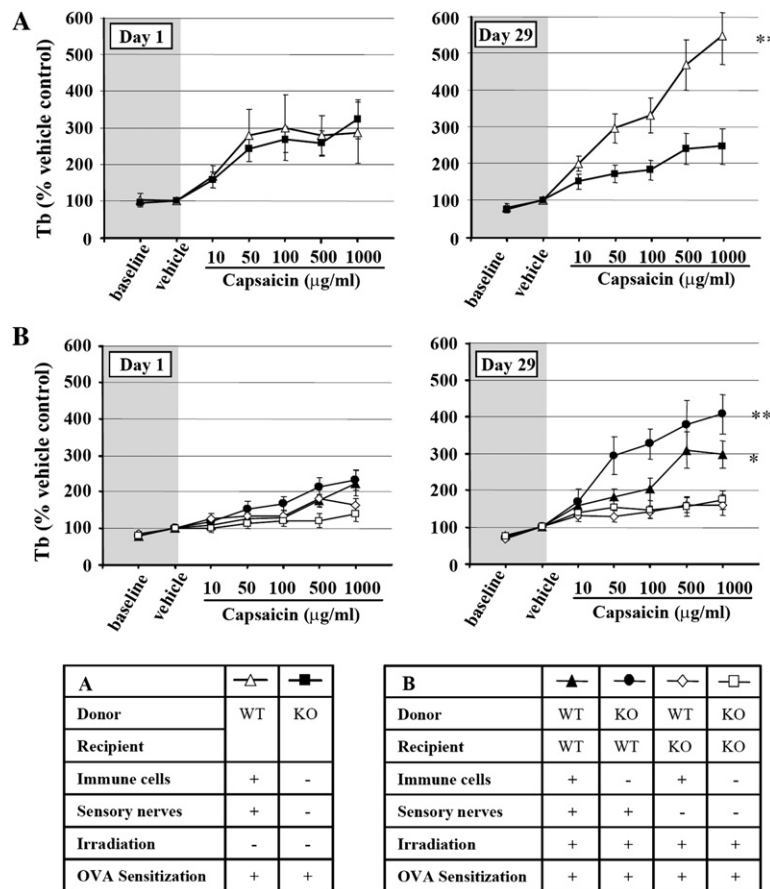


**FIG 1.** Hyperreactivity of sensory nerves to capsaicin. Hyperreactivity of sensory nerves is characterized by enhanced Tb values in response to capsaicin aerosol and was observed in all mice expressing p75NTR in nerves. **A**, Nonirradiated mice. **B**, Chimeric mice. \* $P < .05$  and \*\* $P < .01$  compared with Tb values of the individual animals before sensitization.

role p75NTR plays in allergen-induced hyperreactivity of sensory nerves. Results were compared between individual groups and between values obtained from the same mice measured before OVA sensitization and 24 hours after the last allergen challenge.

Comparison of Tb values from nonirradiated WT (OVA) mice before sensitization and after allergen challenge showed increased Tb values after allergen provocation in response to capsaicin, reflecting the presence of hyperreactivity of the sensory nerves. In contrast, Tb remained unaltered after allergen challenge in the KO (OVA) group (Fig 1).<sup>8</sup>

Irradiation had no effect on sensory nerve function, as indicated by similar Tb values of the nonirradiated WT (OVA) mice and irradiated WT→WT (OVA) mice or nonirradiated KO (OVA) mice and irradiated KO→KO (OVA) mice, respectively. Interestingly, animals of the KO→WT (OVA) group showed sensory nerve hyperreactivity after allergen provocation, as reflected by enhanced Tb values during capsaicin provocation, compared with their respective PBS control groups (see Fig E4 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)) and the Tb values of the individual animals before sensitization.

In contrast, no alterations of Tb values were observed in the WT→KO (OVA) group (Fig 1).

These results suggest that expression of p75NTR in sensory nerves is required for the development of sensory nerve hyperreactivity of the airways.

### Expression of p75NTR on BM-derived cells modulates allergic airway inflammation

Absolute and differential cell counts of BALF cells were performed and cytokine levels (IL-4, IL-5, and IFN- $\gamma$ ) were measured in BALF supernatants to analyze the role of p75NTR expression in immune cells during allergic airway inflammation.

Although total BALF cell numbers were low in all PBS groups and no differences in total BALF cell numbers were observed (data not shown), differential cell counts revealed slightly enhanced lymphocyte numbers in the irradiated PBS groups when compared with the nonirradiated PBS groups (Fig 2).

Allergen provocation led to increased total BALF cell numbers because of an increase in eosinophils, lymphocytes, and neutrophils, but not macrophages, in all OVA-sensitized groups.

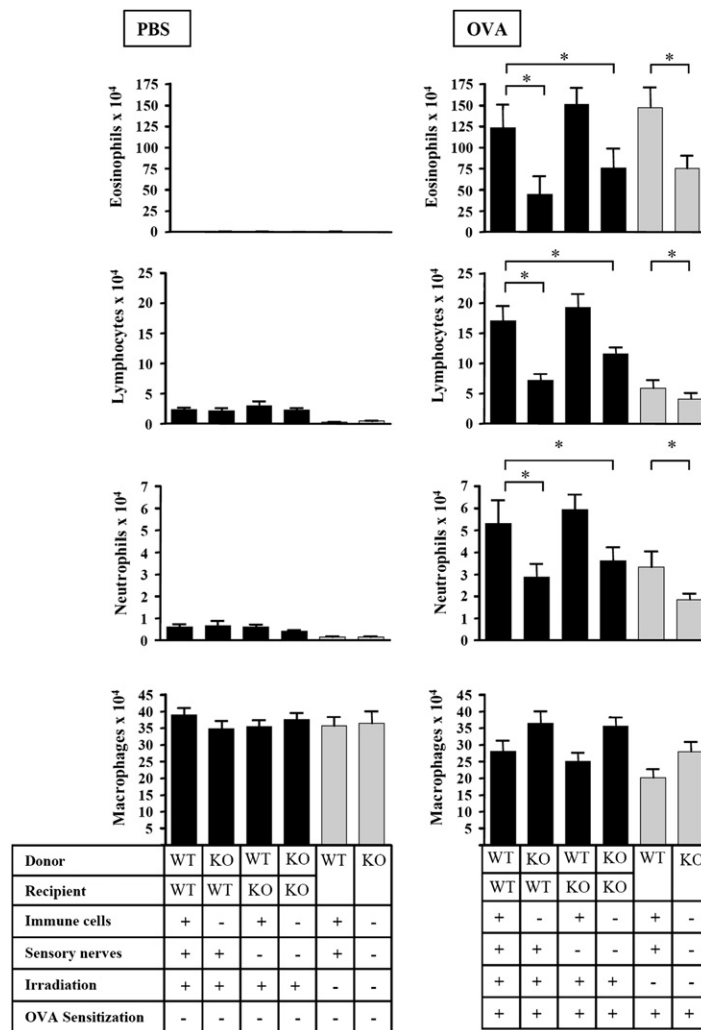


**FIG 2.** Differential cell count in BALF. Increased numbers of eosinophils, neutrophils, and lymphocytes were observed in the BALF of OVA-sensitized animals (OVA group, *right row*) compared with those seen in PBS-treated control mice (*left row*). Mice that received WT BM exhibited higher numbers than recipients of BM from KO mice. \* $P < .05$ .

However, the increase was significantly diminished in nonirradiated KO (OVA) mice compared with that seen in WT (OVA) mice.<sup>8</sup> A similar reduction was seen when WT→WT (OVA) and KO→KO (OVA) mice were compared. A decreased influx of eosinophils, lymphocytes, and neutrophils could also be seen in KO→WT (OVA) mice (Fig 2). Because WT→KO (OVA) mice possess similar numbers of eosinophils, lymphocytes, and neutrophils compared with WT→WT (OVA) mice, airway inflammation seems to depend, at least in part, on p75NTR expression in hemopoietic cells.

To extend on our findings concerning inflammatory cell numbers in the BALF, we also investigated cytokines associated with allergic airway inflammation in BALF. IL-5 levels in BALF supernatants were less than the detection limit in all examined PBS groups. Allergen provocation in all OVA groups led to increased IL-5 levels. However, IL-5 levels were lower in irradiated

mice compared with those in nonirradiated animals (Fig 3). Comparison of IL-5 cytokine levels in nonirradiated WT (OVA) mice and KO (OVA) mice revealed significantly reduced levels in KO (OVA) animals, as previously described.<sup>8</sup> Similar decreases in IL-5 levels could be observed in KO→KO (OVA) compared with WT→WT (OVA) mice. Interestingly, IL-5 levels of KO→WT (OVA) mice were similar to those of KO→KO (OVA) mice and significantly different from those levels observed in WT→WT (OVA) mice. Conversely, WT→KO (OVA) mice possessed IL-5 levels that were not significantly different from those of WT→WT (OVA) mice (Fig 3). The levels of IL-4 and IFN- $\gamma$  were less than the lower limit of quantification in all groups (data not shown).

In conclusion, these results suggest that p75NTR expression on BM-derived cells is involved in the development of allergic airway inflammation.

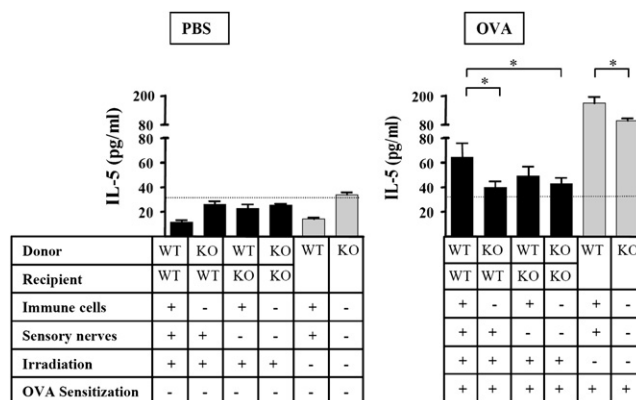


**FIG 3.** IL-5 levels in BALF. Cytokine levels were measured in BALF 24 hours after aerosol challenge. The levels of IL-5 were significantly increased in OVA-sensitized mice compared with those in the appropriate PBS-treated control groups. IL-5 levels were greater in animals that received WT BM. *Dashed line*, Detection limit ( $n = 9-17$  per group). \* $P < .05$ .

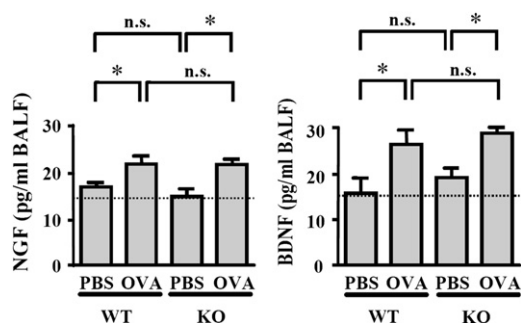


**FIG 4.** Neurotrophin levels in BALF. Neurotrophin levels were measured in BALF 24 hours after aerosol challenge. NGF and BDNF levels were similar in PBS-sensitized WT mice and p75NTR KO animals. OVA aerosol challenge led to increased neurotrophin levels both in WT animals and KO mice. *Dashed line*, Detection limit ( $n = 9-17$  per group). \* $P < .05$ .

## Neurotrophin levels

NGF and BDNF levels were measured in the BALF of WT (PBS), WT (OVA), KO (PBS), and KO (OVA) mice to test the hypothesis that differences in neurotrophin expression levels in KO mice compared with those in WT mice contribute to the development of allergic airway inflammation and hyperreactivity in sensory nerves, such as by means of autocrine p75NTR-dependent neurotrophin synthesis in immune cells.

WT and KO mice exhibited comparable basal neurotrophin levels. Allergen provocation caused a comparable upregulation of both NGF and BDNF levels in KO and WT mice, respectively (Fig 4).

Development of mild airway inflammation in KO mice seems to be sufficient to induce increased neurotrophin levels in the airways. Expression of p75NTR is therefore not required for neurotrophin production. Because neurotrophin release is similar in WT (OVA) and KO (OVA) mice, these data also indicate that differences in hyperreactivity of sensory nerves in WT (OVA) and KO mice

might not be related to differences in neurotrophin production.

Because neurotrophin levels in the nonirradiated WT and KO groups did not show any differences, we did not analyze neurotrophin levels in irradiated mice.

## Relationship between airway inflammation and hyperreactivity of sensory nerves

The presence of airway inflammation was compared with the occurrence of sensory nerve hyperreactivity in OVA-sensitized and OVA-challenged mice to reveal the role of airway inflammation on the development of sensory hyperreactivity and *vice versa* (see Table E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). To answer the question of whether airway inflammation is required to induce hyperreactivity of sensory nerves, we compared those groups in which recipients had WT nerves (recipient = WT mice), and to test the hypothesis that sensory nerve hyperreactivity is required for the development of airway inflammation, we compared those groups in which recipients exhibited intact BM cells (donor = WT mice).

In brief, the presence of sensory nerve hyperreactivity was coupled with the presence of airway inflammation, indicating that airway inflammation might be required for the induction of sensory nerve hyperreactivity. In contrast, airway inflammation seems to be independent of the presence of sensory hyperreactivity. A detailed description is provided in the Results section of the Online Repository at [www.jacionline.org](http://www.jacionline.org).

## DISCUSSION

This study demonstrates that hyperreactivity of sensory nerves in response to allergen seems to depend on p75NTR signaling in sensory nerves. It also provides evidence that the lack of p75NTR in BM-derived immune cells, most likely T lymphocytes, leads to a strongly diminished allergic airway inflammation. Therefore p75NTR ligands, most notably neurotrophins, influence

allergic asthma through 2 independent mechanisms *in vivo* and play an important role in the control of allergic asthma by affecting neuroimmune interaction.<sup>12,16,17</sup>

Previous studies have demonstrated that neurotrophins directly act on immune cells. It has been shown that neurotrophins promote survival and activation of eosinophils, induce differentiation in B lymphocytes, augment cytokine synthesis in T cells, and increase proliferation, differentiation, and production of various mediators in mast cells. However, the effects of neurotrophins on mast cells have been related to Trk receptors rather than p75NTR.<sup>14</sup> Nevertheless, it was unclear what relevance neurotrophins might have in airway inflammation *in vivo*. We could demonstrate that the lack of p75NTR-mediated signaling caused a decrease in IL-5 cytokine production and a concomitant decrease of eosinophil numbers in the BALF. Because p75NTR expression was detected in T cells, but not in eosinophils, our study supports the hypothesis that eosinophil infiltration is caused by T<sub>H</sub>2 cytokines, although the clinical relevance of IL-5 itself has been controversially discussed in the past.<sup>20</sup> However, NGF-transgenic mice, which constitutively overexpressed NGF in the airways, displayed enhanced airway inflammation after allergen provocation, and this inflammation was diminished by application of a dual neurokinin 1/neurokinin 2 receptor antagonist.<sup>21</sup> These data indicate that neurotrophin-induced neurogenic inflammation might be another relevant mechanism causing airway inflammation. In this scenario the cellular sources of neuropeptides have not been firmly identified. However, there is evidence that NGF production within the airways increases the density of tachykinin-producing nerve fibers surrounding the airways.<sup>22</sup> Furthermore, concomitant synthesis of tachykinins by hemopoietic cells has been postulated to contribute to inflammatory responses within the airways *in vivo*, as has been shown in immune complex-mediated and stretch-mediated injuries.<sup>23</sup> Although the relative contribution of neuropeptides has not been investigated in our model, there is evidence that neuropeptides might be involved in neurotrophin synthesis<sup>24</sup> but also that neurotrophins might regulate the production of neuropeptides in immune cells.<sup>25</sup>

Neurotrophin levels were measured in the BALF of WT mice and p75NTR KO mice to test the hypothesis that impaired neurotrophin production *in vivo* might contribute to diminished airway inflammation in p75NTR KO mice. There were no differences between basal NGF and BDNF levels in WT animals or p75NTR KO animals. The allergen-induced increase in NGF and BDNF supports the idea that diminished allergic airway inflammation in p75NTR KO mice is not caused by differences in neurotrophin production but rather is due to the absence of p75NTR-mediated signals in immune cells.

Although our observation that hyperreactivity of sensory nerves in response to capsaicin is mediated by p75NTR agrees with that described by Kerzel et al,<sup>8</sup> our study provides additional evidence for the cellular mechanism underlying the hypersensitivity of pulmonary afferent nerves. We show for the first time that the development of sensory nerve hyperreactivity after allergen

provocation depends on p75NTR signaling in sensory nerves. The mechanisms by which p75NTR mediates sensory nerve hyperreactivity are not completely understood but might include facilitation of Trk-mediated signals.<sup>9,26</sup>

Because our study shows that allergic airway inflammation and hyperreactivity of sensory nerves are 2 entities that can be selectively influenced by p75NTR signaling, and p75NTR expression in the murine lung is restricted to immune cells and airway nerves,<sup>8</sup> we used the BM transfer experiment to further estimate the *in vivo* relevance of neuroimmune interaction in the murine asthma model. Neuroimmune interaction within the lung has been characterized anatomically by intimate associations between various immune cells and different subtypes of neurons.<sup>27,28</sup> Functionally, this might result in bidirectional interactions. Thus it is suggested that immune cell-derived mediators cause depolarization of neurons and decrease their membrane resistance,<sup>29</sup> potentiate transmitter release,<sup>30</sup> and promote survival.<sup>31</sup> Conversely, activated afferent nerve fibers amplify airway inflammation by means of local release of neuropeptides, which then is also termed *neurogenic inflammation*.<sup>7</sup> Neuropeptides promote the chemotaxis, activation, and degranulation of eosinophils; increase lymphocyte proliferation and adhesion; and cause an imbalance in T<sub>H</sub>1/T<sub>H</sub>2-derived cytokines in favor of a T<sub>H</sub>2 profile. Additionally, neuropeptides promote the release of mast cell mediators.<sup>32</sup>

The *in vivo* relevance of neuropeptide-induced neurogenic inflammation in allergic asthma is unclear. Our data indicate that if hyperreactivity of sensory nerves is lacking, the development of airway inflammation after allergen challenge remains unimpaired. These data suggest that airway inflammation in the murine asthma model is probably not caused or modulated by neurogenic inflammation. The importance of neurogenic inflammation has been controversially discussed and might vary between upper and lower airways or species. Stimulation of nasal sensory nerves by capsaicin in patients with allergic rhinitis caused leukocyte influx, albumin leakage, and glandular secretion.<sup>33</sup> However, depletion of sensory nerves in rats followed by allergen sensitization and challenge did not affect the number of inflammatory cells in peripheral blood, the number of eosinophils in lung tissue, or the distribution of eosinophils in the adventitial tissue of blood.<sup>34</sup>

In addition, we could demonstrate that the presence of sensory nerve hyperreactivity was always coupled with airway inflammation, which supports the hypothesis that airway inflammation is required for the development of sensory nerve hyperreactivity. The underlying mechanisms have not yet been characterized in detail. Although it has been shown that vagal C-fiber afferents in lungs can be stimulated by exogenously added eosinophil cationic protein eliciting pulmonary chemoreflex responses,<sup>11</sup> our data suggest that neurotrophins might be critically involved in the development of sensory nerve hyperreactivity *in vivo*. Thus the selective lack of p75NTR in neurons prevented the development of sensory nerve hyperreactivity, even when severe airway inflammation was present. Interestingly, mild airway inflammation was sufficient to

induce distinct hyperreactivity of sensory nerves. In animals with mild and severe airway inflammation, the number of BALF cells and cytokine levels were different. However, neurotrophin levels were similar, as indicated by comparable neurotrophin levels in WT mice exhibiting severe airway inflammation and p75NTR KO mice exhibiting mild airway inflammation after allergen challenge. These results support the hypothesis that neurotrophins play a crucial role in the development of sensory nerve hyperreactivity.

In conclusion, our study provides evidence for a dual role of p75NTR in allergic asthma *in vivo*. p75NTR participates in mediating neurotrophin signals to immune cells, as well as to nerve cells. Furthermore, it supports the hypothesis that immune cells influence sensory nerve function *in vivo*. This immunoneuronal interaction might be involved in the pathogenesis of allergic asthma.

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