

ORIGINAL PAPER

Airway hyper-responsiveness in allergic asthma in guinea-pigs is mediated by nerve growth factor via the induction of substance P: a potential role for trkA

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Summary

Background: The neurotrophin nerve growth factor (NGF) has been implicated as a mediator in allergic asthma. Direct evidence that inhibition of NGF-induced activation of neurotrophin receptors leads to improvement of airway symptoms is lacking. We therefore studied the effects of inhibitors of NGF signal transduction on the development of airway hyper-responsiveness (AHR) and pulmonary inflammation in a guinea-pig model for allergic asthma.

Methods: Airway responsiveness to the contractile agonist histamine was measured *in vivo* in guinea-pigs that were sensitized and challenged with ovalbumin (OVA). Inflammatory cell influx and NGF levels were determined in bronchoalveolar lavage fluid (BALF). Substance P, a key mediator of inflammation, was measured in lung tissue by radioimmunoassay, while substance P immunoreactive neurons in nodose ganglia were measured by immunohistochemistry.

Results: OVA challenge induced an AHR after 24 h in OVA-sensitized guinea-pigs. This coincided with an increase in the amount of NGF in BALF. Simultaneously, an increase in the percentage of substance P immunoreactive neurons in the nodose ganglia and an increase in the amount of substance P in lung tissue were found. We used tyrosine kinase inhibitors to block the signal transduction of the high-affinity NGF receptor, tyrosine kinase A (trkA). Treatment with the tyrosine kinase inhibitors (K252a or tyrphostin AG879) both inhibited the development of AHR, and prevented the increase in substance P in the nodose ganglia and lung tissue completely whereas both inhibitors had no effect on baseline airway resistance. Neither treatment with K252a or tyrphostin AG879 changed the influx of inflammatory cells in the BALF due to allergen challenge.

Conclusions: We conclude that substance P plays a role in the induction of AHR in our model for allergic asthma which is most likely mediated by NGF. As both tyrosine kinase inhibitors AG879 and K252a show a similar inhibitory effect on airway function after allergen challenge, although both tyrosine kinase inhibitors exhibit different non-specific inhibitory effects on targets other than trkA tyrosine kinases, it is likely that the induction of substance P derived from sensory nerves is mediated by NGF via its high-affinity receptor trkA.

Keywords asthma, bronchoconstriction, NGF, substance P and airway inflammation

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Introduction

Nerve growth factor (NGF) is a member of the neurotrophin family of proteins that can regulate neuronal development, maintenance, and recovery from injury. It now becomes clear that NGF also plays a crucial role in inflammation [1, 2]. Several inflammatory mediators have been shown to induce the release of NGF [3, 4]. In addition to neurons, non-neuronal cells also are able to synthesize NGF [3, 5–11]. As allergic asthma is characterized by an airway inflammation [12], NGF has become a well-studied mediator in this disease (see for recent reviews [13–15]). Neurotrophins are up-regulated in allergic asthma and are involved in allergen-induced bronchoconstriction and airway hyper-responsiveness (AHR) [16–21]. Thus far, it is not clear how NGF mediates direct bronchoconstriction as well as AHR. In this study, we address the potential mechanisms behind the effects caused by NGF in allergic asthma.

NGF is able to affect neuronal function and induce an enhanced production of substance P and other tachykinins [22]. Tachykinins play a role in neurogenic inflammation and in the development of AHR and asthma [23, 24]. In a previous study, we have demonstrated that administration of NGF to guinea-pigs results in AHR, which is a hallmark of the asthmatic constitution, which is mediated by the receptor for substance P, the neurokinin receptor 1 [16].

Taken together, this indicates that NGF may play a role in allergic reactions in the airways. This prompted us to investigate whether NGF is indeed involved in the development of AHR and pulmonary inflammation in allergic asthma. NGF can interact with two receptors: either the tyrosine kinase A (trkA), binding NGF with high affinity, or the low-affinity receptor p75, which can also bind brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and NT-4. Of these receptors only trkA uses tyrosine kinases for its signal transduction (reviewed in Lindsay and Harmer [25]).

Here, we tested the possible role of the high-affinity receptor for NGF trkA on ovalbumin (OVA)-induced AHR and influx of inflammatory cells into the lungs in sensitized guinea-pigs by using tyrosine kinase inhibitors. Furthermore, we measured levels of NGF in bronchoalveolar lavage fluid (BALF) as we hypothesize an increase in NGF due to the allergic response in the airways. As we predicted an augmented release of substance P, we determined the number of substance P-containing nerves in the nodose ganglia as well as the amount of substance P in the lung tissue.

Materials and methods

Animal model: experiment 1

All animal protocols described in this study were approved by the Utrecht University Committee for Animal

Experimentation. Male Duncan Hartley guinea-pigs (weighing approximately 400 g; Harlan CPB, Zeist, the Netherlands) were sensitized to OVA, grade V (Sigma, St. Louis, MO, USA), by injections of a gel containing 20 µg OVA/mL, and 200 mg Al(OH)₃/mL as an adjuvant. Six injections were administered: one injection of 0.5 mL intraperitoneally (i.p.), one of 0.1 mL nuchally, two injections of 0.1 mL axillary, and two of 0.1 mL inguinally. Fourteen days after the sensitization procedure, the guinea-pigs were challenged with OVA. The animals received an intraperitoneal injection with the histamine H₁ receptor antagonist, pyrilamine (10 mg/kg body weight; Sigma), 30 min before allergen challenge, to prevent asphyxial collapse. Each challenge consisted of a nebulization of 0.01% OVA in sterile saline (Ultra-Neb 2000™, Devilbiss, Sommerset, PA, USA) for 1 min in a box in which the animals had been placed individually. Control animals were not sensitized and received a vehicle gel and subsequently challenged with OVA. Each group consisted of $n = 6$.

Alternative negative control animals: experiment 2

To ensure that our data were reproducible, we performed a second, separate animal experiment. Experiment 2 ($n = 3–5$) is the same as experiment 1 with one difference: we used an alternative negative control in experiment 2. In experiment 2, negative control animals were sensitized with OVA and subsequently nebulized with saline. Airway resistance curves (see 'lung function') did not differ for the negative controls (curves of both experiments were compared using a two-way repeated-measures ANOVA $F_{(1,54)} = 1.03$, $P = 0.407$). As there were no differences between the negative controls in experiments 1 and 2, we combined the airway function data in Fig. 1.

Alternative sensitization protocol to determine nerve growth factor levels: experiment 3

For the measurements of NGF in the BALF and subsequently substance P in neurons and lung tissue, a separate group of female guinea-pigs (200–250 g body weight, Charles River Wiga, Kreisllegg, Germany) was used. We thought a separate experiment necessary in order to avoid an interfering effect of measuring airway constriction 24 h before on NGF levels. For experiment 3, the following sensitization procedure was used. The guinea-pigs were sensitized by an injection of 1 mL containing 10 mg OVA and 50 mg Al(OH)₃ i.p. at days 1, 14, and 28. Control animals received the same injections without OVA. At day 35, sensitized and control animals were exposed to the allergen by nebulization of 10 mL OVA solution (0.1%) for 60 min in a chamber (pari-boy type 37.00, pari-Werk GmbH, Starnberg, Germany; [26]).

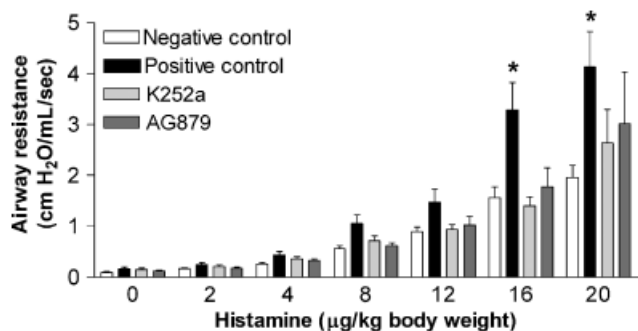


Fig. 1. Effect of tyrosine kinase inhibitors K252a and tyrphostin AG879, administered 1 h before and 6 h after allergen challenge, on airway hyper-responsiveness (AHR) 24 h after allergen challenge. Airway responsiveness was measured *in vivo* in guinea-pigs upon administration of increasing doses of histamine. Negative control, no ovalbumin (OVA) response evoked, vehicle treated; K252a and AG879, OVA sensitized and challenged, K252a or tyrphostin AG879 treated; positive control, OVA sensitized and challenged; $n=8-11$. * $P < 0.05$ positive vs. negative control, Bonferroni's *post hoc* test.

Treatment with tyrosine kinase inhibitors

One hour before and 6 h after the OVA challenge, the OVA-sensitized guinea-pigs were treated with tyrosine kinase inhibitors or their vehicles. The control animals were treated with 100 µL saline (experiments 1 and 2).

Two different tyrosine kinase inhibitors were used: K252a (Calbiochem Novabiochem, Läufelfingen, Switzerland) and tyrphostin AG879 (Calbiochem Novabiochem). Both are inhibitors of the tyrosine kinases on the trkA [27, 28]. Both were dissolved in saline containing 2% dimethylsulphoxide (DMSO). We applied 100 µL of 0.1 mM K252a or 200 µL of 0.2 mM tyrphostin AG879 intranasally, whereas saline containing 2% DMSO was used as a control.

In order to apply the solutions intranasally, the guinea-pigs were anaesthetized using halothane (2% halothane, 0.4 L O₂/min, 2 L N₂O/min, approximately 15–30 s). The animals all regained consciousness within 30 s after this procedure.

Lung function measurement

The airway resistance was measured 24 h after the OVA challenge in the anaesthetized guinea-pigs upon intravenous administration of increasing doses of histamine (OPG, Zeist, the Netherlands) or methacholine (Janssen Chimica, Beerse, Belgium). To this extent, guinea-pigs were anaesthetized with urethane (2 g/kg body weight i.p.). Lung function was measured in spontaneously breathing guinea-pigs, essentially after Amdur and Mead [29]. Air flow and tidal volume were determined by cannulating and connecting the trachea via a Fleisch flow head (Meijnhart, Bannik, the Netherlands) to a pneumo-

tachograph. A pressure transducer (MP45-2; Validyne Engineering Corp., Northridge, CA, USA) measured the transpulmonary pressure by the difference between the tracheal cannula and an oesophageal cannula. In this way, airway resistance was determined breath by breath by dividing transpulmonary pressure by air flow at isovolumetric points. Airway resistance values were averaged per three breaths and are presented as actual value. A small polyethylene catheter (PE-50, Maxxim Medical Europe BV, Pen Basch, the Netherlands) was placed in the right jugular vein for intravenous administration of histamine.

Bronchoalveolar lavage

After completion of the dose-response curve, the lungs were lavaged three times through the tracheal cannula with 10 mL aliquots of warmed (37 °C) saline. The total amount of BAL cells was counted using a Burkert-Türk chamber. For differential cell counts, cytospin preparations were stained with Diff-Quick (Merz & Dade, Dudingen, Switzerland). Cells were identified as mononuclear cells, lymphocytes, neutrophils and eosinophils by standard morphology.

NGF was measured in BALF obtained 24 h after allergen challenge using a commercial ELISA kit from R&D Systems (Abingdon, UK) using an anti-human NGF detection antibody.

Immunohistochemistry

Nodeose ganglia were obtained from the guinea-pigs 24 h after allergen challenge directly after finishing lung function measurement for experiment 1, or 24 h after allergen challenge for experiment 3. Zamboni-fixed sensory ganglia were rinsed in 0.1 M phosphate buffer and cryoprotected with 18% sucrose in phosphate buffer overnight. Sections of the sensory ganglia were cut on a cryostat (500 OM Microm, Waldorf, Germany) at 10 µm and air dried for 30 min. Sections were incubated with a blocking solution containing 1% bovine serum albumin and 10% normal swine serum in phosphate buffer for 60 min, followed by an overnight incubation with a monoclonal rat antibody against substance P (1 : 1000, Boehringer Ingelheim, Alkmaar, the Netherlands). After washing in phosphate-buffered saline, a biotinylated anti-rat Ig from sheep (Amersham, dilution 1 : 50) was applied for 1 h. After several washes in phosphate buffered saline, sections were incubated with a mixture of streptavidin-Texas-Red-conjugate (Amersham, Diegem, Belgium, dilution 1 : 50). Slides were washed thoroughly in phosphate-buffered saline and covered with coverslips with buffered glycerol (pH 8.6). For microscopic evaluation, slides with sections of the sensory ganglia were coded, to ensure that the investigator could not identify the tissues with respect to sensitization status. Randomly,

two sections were chosen and in each section 100 neurons were examined for their substance P immunoreactivity, using a square ocular grid.

Radioimmunoassay

Lung samples were obtained at the same moment, from the same animals, as sensory ganglia. Samples of peripheral lung were snap frozen in liquid nitrogen. Substance P radioimmunoassay was performed as described previously [30]. Non-radioactive synthetic peptides were obtained from Peninsula and radioactively labelled peptides from Amersham.

Statistics

Means and standard error of the mean (SEM) were calculated. For airway function measurement, a two-way ANOVA with repeated measures was used followed by Bonferroni's *post hoc* tests. *P*-values were determined using a two-tailed unpaired Student's *t*-test for the comparison of two means, unless stated otherwise.

Results

Lung function

Experiments 1 and 2: AHR to histamine was found 24 h after the OVA challenge in OVA-sensitized guinea-pigs as compared with non-sensitized animals. A two-way ANOVA showed that the curves for all groups (controls, K252a, and AG879) were different ($F_{(3, 204)} = 4.38$; $P = 0.0104$). The ANOVA also showed an interaction between treatment and histamine, which indicates that the treatment had a different effect depending on the dose of histamine ($F_{(18, 204)} = 2.40$; $P = 0.017$). *Post hoc* testing revealed that the OVA-sensitized animals showed an increased responsiveness at the histamine dose of 16 µg/kg and all higher doses ($P < 0.05$, Bonferroni's *post hoc* test; Fig. 1). Treatment with K252a or tyrphostin AG879 could prevent the hyper-responsiveness to histamine after OVA challenge, as Bonferroni's *post hoc* testing revealed no difference in airway resistance at any concentration of histamine. This was confirmed by a two-way ANOVA for either negative control and K252a as well as the negative control and AG879, which showed the curves were equal ($F_{(1, 108)} = 0.8291$; $P = 0.3746$; and $F_{(1, 102)} = 1.245$ $P = 0.2800$, respectively). Thus, the animals treated with these tyrosine kinase inhibitors did not show a significant increase in airway responsiveness at any concentration of histamine (Fig. 1).

Substance P

Experiment 1. An increase in substance P was found in the guinea-pigs, which were used for lung function

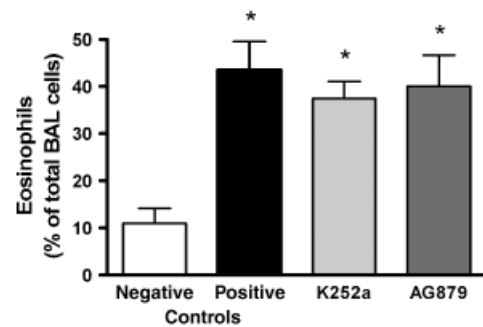


Fig. 2. The percentage of eosinophils of the bronchoalveolar lavage (BAL) cells did not change 24 h after allergen challenge by tyrosine kinase inhibitor treatment. Treatment with vehicle (positive control), K252a or tyrphostin AG879 took place 1 h before and 6 h after allergen challenge. * $P < 0.001$ all groups vs. negative control.

measurements. The percentage substance P immunoreactive neurons in the nodose ganglia increased from 24.6 ± 1.8 to 46.0 ± 2.5 ($P < 0.01$) for vehicle and positive control, respectively (Fig. 3). The substance P content in the lung increased from 250 ± 33 to 484 ± 12 pmol/g wet weight ($P < 0.04$) for vehicle control and positive control, respectively (Fig. 4).

Experiment 3. Changes in substance P immunoreactive neurons as well as lung content were similar when using an alternative sensitization protocol, as used in experiment 3. The percentage of substance P immunoreactive neurons in the nodose ganglia was augmented in sensitized animals as compared with non-sensitized animals 24 h after allergen challenge: from $22 \pm 2\%$ to $43 \pm 4\%$ ($P < 0.0003$). At the same time, substance P content of lung tissue was increased as well: from 216 ± 17 to 472 ± 41 pmol/g wet weight ($P < 0.0002$).

The guinea-pigs that were treated with the tyrosine kinase inhibitors revealed a complete inhibition of the increase in the percentage of substance P immunoreactive

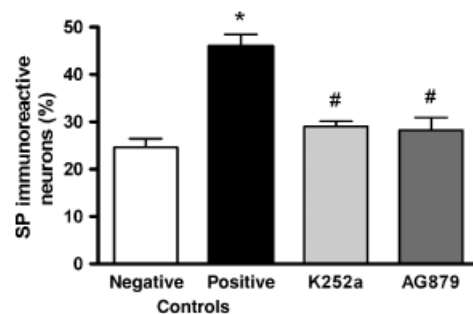


Fig. 3. The percentage of substance P immunoreactive neurons in nodose ganglia increased after allergen challenge and this increase was completely blocked by tyrosine kinase inhibitor treatment. Treatment with vehicle, K252a and tyrphostin AG879 took place 1 h before and 6 h after allergen challenge. All groups $n = 4-5$. * $P < 0.01$ positive controls vs. negative controls, # $P < 0.01$ K252a and AG879 vs. positive control.

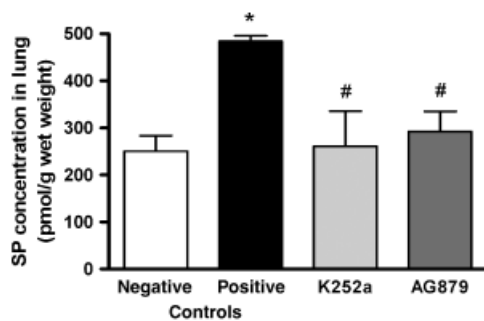


Fig. 4. Substance P concentration in the lung tissues increased after allergen challenge. This increase was completely prevented by tyrosine kinase inhibitor treatment. Treatment with vehicle, K252a and tyrphostin AG879 took place 1 h before and 6 h after allergen challenge. All groups $n = 5$. * $P < 0.004$ positive controls vs. negative control, # $P < 0.02$ K252a and AG879 vs. positive control.

Table 1. Number of cells in bronchoalveolar lavage fluid

	Ovalbumin sensitization	Ovalbumin challenge	Total cell count (in 10^6) in bronchoalveolar lavage fluid
Negative control	—	+	19.4 ± 3.4
Positive control	+	+	$65.3 \pm 19.3^*$
K252a	+	+	$44.9 \pm 4.1^*$
AG879	+	+	$46.6 \pm 11.7^*$

* $P < 0.03$ vs. negative control.

neurons: from 46.0 ± 2.5 to 29.0 ± 1.1 for K252a and to $28.2 \pm 2.7\%$ for tyrphostin AG879 ($P < 0.01$, Fig. 3). A similar decrease was seen when measuring substance P content in the lung tissue: from 484 ± 12 to 262 ± 74 for K252a and to 292 ± 43 pmol/g wet weight for tyrphostin AG879 ($P < 0.02$, Fig. 4).

Bronchoalveolar lavage

Experiment 1. The AHR coincided with an influx of cells in the BALF (Table 1). The percentage of eosinophils increased in OVA-sensitized animals as compared with non-sensitized animals due to allergen challenge (from $11 \pm 3.2\%$ in non-sensitized to $43.6 \pm 6.0\%$ in sensitized animals, $P < 0.001$, Fig. 2). Neither the total amount of cells in the BALF (Table 1) nor the percentage of eosinophils was affected by treatment with K252a or tyrphostin AG879 (Fig. 2). The percentages of lymphocytes, neutrophils, and macrophages were not affected by treatment with K252a or tyrphostin AG879 (data not shown).

Experiment 3. NGF levels were increased in BALF in sensitized animals as compared with non-sensitized animals 24 h after allergen challenge (Fig. 5). NGF levels increased from 22.6 ± 2.6 to 34.5 ± 6.0 pmol/mL ($P < 0.03$, one-tailed Student's *t*-test).

We used a separate group of guinea-pigs (experiment 3) to collect BAL to measure NGF, whereas we measured changes in substance P in BAL derived from experiment 1. Therefore, we wanted to ensure that similar changes in substance P parameters were found in experiments 1 and 3 (which differ slightly in their sensitization protocol) in order to speculate about presumed NGF changes in BALF in experiment 1. Indeed, the number of substance P immunoreactive neurons as counted in the ganglia was similar to our other experimental animals (see 'Substance P').

Discussion

In this study, we used a model for allergic asthma in OVA-sensitized guinea-pigs, that shows increased airway responsiveness [31]. The OVA challenge induced NGF release in the airways, substance P increase in the lung tissue as well as in the nodose ganglia coinciding the predicted AHR and cellular influx (Figs 1–5). Inhibition of tyrosine kinases by tyrosine kinase inhibitors K252a and tyrphostin AG879 inhibited the development of AHR in this model for allergic asthma (Fig. 1). Both tyrosine kinase inhibitors are claimed to have a specific inhibitory effect on the trkA tyrosine kinases. However, both tyrosine kinase inhibitors exhibit different non-specific effects on targets other than trkA tyrosine kinases. As both inhibitors show a similar effect on airway function after allergen challenge, we presume that this is mediated via trkA. As the induction of substance P is an effect that has been repeatedly shown to be dependent upon NGF [25, 32, 33], the conclusion that the effects reported here are mediated by NGF via trkA is strengthened by the fact that both K252a as well tyrphostin AG879 prevent AHR as well as reduce the level of substance P in the lung and the number of neurons substance P immunoreactive neurons in the nodose ganglia (Figs 3 and 4). We conclude that OVA-induced AHR is mediated in part by NGF via induction of substance P presumably by the NGF high-affinity receptor trkA.

Histamine will specifically induce release of increased amounts of substance P in the lung after OVA challenge, as the guinea-pigs were challenged by OVA-containing aerosol (i.e. in the airways). The inhalation of OVA induces an increase in substance P in ganglia which project to the airways (Fig. 3). Substance P is a contractile neuropeptide in the airways and induces AHR to intravenous histamine administration in guinea-pigs [31]. Stimulation with contractile agents like histamine induces a contraction as well as the release of several mediators among which substance P [34], which in its turn will contribute to the airway resistance itself as well as induce the release of histamine in the airways with subsequent contractile effects [35].

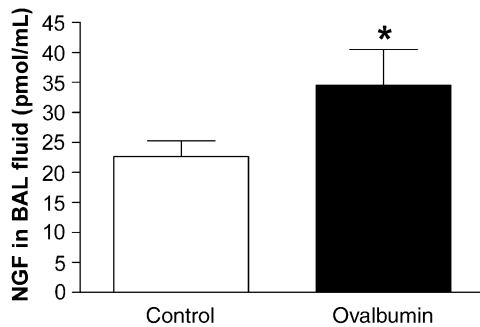


Fig. 5. The amount of nerve growth factor (NGF) was elevated in bronchoalveolar lavage fluid (BALF) after ovalbumin (OVA) challenge in OVA-sensitized guinea-pigs (* $P < 0.03$, one-tailed Student's *t*-test).

Guinea-pigs sensitized to OVA showed an AHR to histamine 24 h after allergen challenge (Fig. 1). Inhibition of the trkA tyrosine kinases by the claimed specific tyrosine kinase inhibitors K252a and tyrphostin AG879 inhibited the development of AHR (Fig. 1). Unfortunately, K252a and tyrphostin AG879 inhibit, although to lesser extent, the phosphorylation of the neurotrophin receptors trkB and trkC [36–38] in addition to trkA. Besides this, both K252a and tyrphostin AG879 are known to inhibit other tyrosine kinases than merely trkA tyrosine kinases. K252a is known to inhibit CamKII, calmodulin, MCLK, PKA as well as PKC [39, 40]. Tyrphostin AG 879 inhibits ErbB2, FLK-1 as well as FAK besides its main action on trkA tyrosine kinases [41]. Tyrosine kinase is a signalling molecule in cascades activated by multiple growth factors, although some data suggest a relative selectivity of K252a for the trkA-associated tyrosine kinase but not for kinases mediated by EGF, insulin, and v-src [37]. As both tyrosine kinase inhibitors, which both exhibit different non-specific effects on targets other than trkA tyrosine kinases show a similar effect on airway function after allergen challenge, we still presume this is mediated via trkA. This conclusion is strengthened by the fact that both K252a as well as tyrphostin AG879 affect airway function as well as substance P. The latter is reported to be associated to trkA activation [25].

It is well documented that the non-specific tyrosine kinase inhibitors genistein and tyrphostin 47 have no effect on airway resistance after histamine or methacholine administration in guinea-pigs [42, 43]. This is in contrast to the situation with antigenic challenge where tyrosine kinase inhibitors can affect bronchoconstriction [42–44]. In our study, basal airway resistance was not affected by previous administration of tyrosine kinase inhibitors. Taken together with the fact that histamine induces smooth muscle contraction via the H1 receptor, which does not signal via tyrosine kinases [42, 43, 45], we are confident that tyrosine kinases alone did not alter airway resistance after histamine challenge in our model.

Apparently, the inhibition of the down-stream signalling of the trkA receptor was effectively preventing induction of AHR. The importance of the trkA in long-term inflammatory processes is confirmed by the enhanced numbers of trkA immunoreactive neurons in a model for inflammatory chronic pain [46]. We conclude that the tyrosine kinases, most likely those of the trkA receptor, are involved in the development of AHR in our model for allergic asthma.

The cellular influx in the airways in this allergic asthma model was not affected by interfering with tyrosine kinase inhibitors whose main target is trkA (Table 1 and Fig. 2). Therefore, the possible involvement of NGF in the airway function of allergic guinea-pigs can be envisioned as being distal to inflammatory cell influx into the lining fluid of the airways. Future experiments studying infiltration of inflammatory cells into the airway tissue could reveal another role for infiltrating inflammatory cells.

NGF is the neurotrophic factor with the highest binding affinity for trkA [47]. Interestingly, the development of AHR in this model of allergic asthma coincided with an increase in NGF levels in BALF 24 h after allergen challenge (Fig. 5). NGF increases have been reported before in models for allergic airway diseases in mice [48] as well as in patients with allergic asthma or allergic rhinitis (AR) [12, 48, 49].

Possibly, NGF originates from the infiltrating inflammatory cells. Indeed, NGF formation has been demonstrated in various immune organs including the spleen, lymph nodes, and thymus, and immune cells such as mast cells, eosinophils, and B and T cells [50]. It is unknown which cells are the major source for NGF in the airways. Sanico et al. [12] show a rapid increase in NGF in nasal lavage fluids shortly after allergen provocation in patients with allergic rhinitis. The authors suggest that the mast cell is the most likely source of NGF. Other than infiltrating cells, epithelial cells as well as fibroblasts are a potential source of NGF as well [51, 52].

NGF acts on a variety of cells of the immune system [2], including mast cells, eosinophils, and B and T lymphocytes [53]. Inhibiting trkA signalling did not affect the number of cells; however, it could have affected the activity of the cells in the airways and thus lowering the pathologic effects of the allergic inflammation. For instance, mast cells' function could have been altered as mast cells express trkA [54] and NGF has been shown to induce phenotypic switching of the mast cells [55] and change the cytokines that are expressed [56] as well as increase their number [57]. NGF can activate eosinophils [7] as well as T cells [6] via trkA. Therefore, the tyrosine kinase inhibitors could have affected their activation state. Furthermore, there is a role for NGF as an autocrine survival factor that rescues macrophages [10].

As shown before, guinea-pigs sensitized to and challenged with OVA show an increase in substance P immunoreactive neurons in the nodose ganglia [26, 58]. In the present study, a similar increase was found in substance P immunoreactive neurons in the nodose ganglia as well as an increase in substance P content in the lung tissue (Figs 3 and 4). There was a correlation between increase in NGF in BALF and substance P increase in lung tissue in the same animals ($r^2 = 0.625$, $P < 0.02$, $n = 8$). Therefore, it is likely that NGF is the important mediator in inducing increases in substance P. This is confirmed by Hunter et al. [59], who showed that tracheal application of NGF can increase the percentage of substance P immunoreactive neurons in nodose ganglia. Substance P-containing fibres in guinea-pig airways are C-fibres, whereas neurons with A δ -fibres normally do not contain substance P. NGF induced the formation of substance P in nodose neurons with A δ -fibres [58, 59]. The increase is similar to the increase in substance P in nodose ganglion neurons with A δ -fibres in allergic models [26, 58, 59].

We studied the nodose ganglia, which might give an underestimate of our findings, as more (substance P positive) sensory neurons that are airway specific are found in the jugular ganglia than in the nodose [59, 60]. At the same time, NGF induces a larger increase in substance P-positive neurons in the nodose than in the jugular ganglia [59]. We, therefore, think that the increase as well as inhibition of this increase in substance P positive neurons in the nodose ganglia gives a good reflection of what is happening in airway-specific sensory neurons.

Interestingly, the tyrosine kinase inhibitors inhibited the increases in substance P immunoreactive neurons and substance P content in the lung tissue (Figs 3 and 4). In a model using toluene diisocyanate (TDI) to induce airway inflammation, the tyrosine kinase inhibitor K252a was capable of reversing the induction in substance P in a similar fashion as we showed here [61]. Furthermore, increases in substance P, together with induction of AHR have been reported before; for instance in models using TDI [62], ozone [63] or citric acid [24] to induce changes in airway function. Our group has shown a role for tachykinins in the IL-5 [64] and 13-hydroxyoctadecadienoic acid- [65] induced AHR in animal models. Moreover, tachykinin levels are elevated in patients with asthma [23], and protective effects of neurokinin receptor antagonists have been reported [66].

Additional proof that points in the direction of trkA-mediated substance P induction is provided by a recent study, which shows an increase in substance P as well as trkA in large-diameter neurons in the nodose and jugular ganglia after injection of NGF in the lower airways of mice [67]. This suggests that there may be trkA-mediated substance P induction to induce neurogenic airway inflammation [67].

Concluding remarks

NGF is up-regulated in our guinea-pig model after allergen challenge (Fig. 5) coinciding with an AHR (Fig. 1). As we showed before, NGF administration induces AHR [16]. Here, we found a simultaneous increase in substance P 24 h after allergen challenge in our model for allergic asthma (Figs 3 and 4). We conclude that NGF plays a role in the induction of AHR, through substance P derived from sensory nerves in our model for allergic asthma. The induction of substance P seems to be due to activation of the high-affinity receptor for NGF trkA, as both tyrosine kinase inhibitors K252a and tyrphostin AG879 were able to prevent allergen induced AHR as well substance P induction. This study provides evidence for a reduction of the pathologic effects in our model for allergic asthma that could be mediated by NGF via trkA receptor activation.

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