The role of the tachykinin NK_1 receptor in airway changes in a mouse model of allergic asthma

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Background: Tachykinins are present in sensory nerves and in nonneuronal cells like macrophages. Human data suggest a role for these peptides in asthma, but the exact role of tachykinins and their receptors in allergic airway inflammation is still a matter of debate.
Objective: The aim of this study was to determine the role of the tachykinin NK_1 receptor in allergic airway responses in a mouse model.
Methods: Tachykinin NK_1 receptor wild-type and knockout animals were sensitized intraperitoneally to ovalbumin and subsequently exposed from days 14 to 21 to aerosolized ovalbumin (1%). On day 22, the immunologic and histologic changes were evaluated, and lung function measurements were performed.
Results: Mice lacking the tachykinin NK_1 receptor and their wild-type litter mates developed inflammatory cell infiltrates in the airways and ovalbumin-specific IgE on sensitization and exposure to ovalbumin compared with saline-exposed controls. No differences were detected between wild-type and knockout mice. The substance P content of alveolar macrophages was not influenced by ovalbumin or by the lack of the NK_1 receptor. Ovalbumin-induced hyperresponsiveness was not observed, but at baseline, the knockout mice were more reactive despite similar morphology. Ovalbumin induced more goblet cell hyperplasia in wild-type animals compared with knockout animals. No differences in airway wall thickness were observed.
Conclusion: These data suggest that tachykinin NK_1 receptors do not affect allergic airway inflammation or endogenous substance P content of alveolar macrophages but influence baseline responsiveness and promote features of remodeling such as goblet cell hyperplasia. (J Allergy Clin Immunol 2004;113:1093-9.)

Key words: *Substance P, hyperplasia, goblet cells, knockout mice*

Abbreviations used
Ao: Area defined by the adventitial perimeter
Abm: Area defined by the basement membrane
BAL: Bronchoalveolar lavage
Pbm: Length of basement membrane
UK: United Kingdom
WT: Wild-type

The tachykinins, substance P, and neurokinin A are present in sensory afferent nerves and inflammatory cells in the airways. They may be released by a variety of stimuli (eg, allergen, ozone) and have various effects including smooth muscle contraction; facilitation of cholinergic neurotransmission; submucosal gland secretion; vasodilatation; increase in vascular permeability; stimulation of mast cells, B and T lymphocytes, and macrophages; chemoattraction of eosinophils and neutrophils; and the vascular adhesion of neutrophils.[1]

Tachykinins mediate their effects by stimulation of tachykinin NK_1, NK_2 and NK_3 receptors.[2] NK_1 receptors are mainly involved in neurogenic inflammation (microvascular leakage and mucus secretion), whereas NK_2 receptors are considered to be important in airway smooth muscle contraction. NK_3 receptors have not been detected in the airways yet, despite emerging functional evidence for a role in inflammation and responsiveness.

Several lines of evidence indicate a role for tachykinins in airway diseases such as asthma. Elevated levels of tachykinins have been recovered from the airways of patients with asthma, and airway inflammation leads to an upregulation of tachykinin NK_1 and NK_2 receptors.[1] In guinea pigs, the tachykinin NK_1 receptor is involved in both the antigen-induced airway hyperresponsiveness to histamine and the infiltration of inflammatory cells.[3] Substance P also exerts several proinflammatory actions on macrophages[4-7]; even an autocrine function of this peptide has been suggested.[8] Because alveolar macrophages can be considered the first line of defense against inhaled particular matter and microorganisms, this effect may also contribute to the pathology of asthma.

However, from animal studies, there is still debate about the precise role of the tachykinin NK_1 receptor in allergic inflammation. An involvement of the tachykinin NK_1 receptor in allergic airway inflammation could not been detected in the Brown Norway rat model,[9] and other studies in guinea pigs demonstrated no influence of the tachykinin NK_1 receptor on antigen-induced airway eosinophilia[10] and cell influx in bronchoalveolar lavage (BAL).[11]

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Therefore, the aim of our study was to point out the role of the tachykinin NK_1 receptor in antigen-induced airway inflammation, responsiveness, and structural changes by using tachykinin NK_1 receptor knockout mice, which is, compared with antagonist studies, a strong model to evaluate involvement of receptors. Furthermore, we wanted to evaluate the role of the NK_1 receptor in controlling the endogenous substance P production of alveolar macrophages during inflammatory stress.

METHODS

Mice

Tachykinin NK_1 receptor knockout and wild-type (WT) mice were derived as described from the mating of heterozygous tachykinin NK_1 receptor mice.[12] The tachykinin NK_1 receptor knockout and WT breeding pairs were provided by the lab of S. Hunt (Cambridge, United Kingdom [UK]). The animals were bred locally and maintained under germ-free conditions in a conventional animal house in the animal research facilities of the Faculty of Medicine and Health Sciences, Ghent University Hospital, and received food and water ad libitum.

Immunization and exposure of mice

On day 0, all mice where actively immunized with ovalbumin (grade III; Sigma Chemical Co, Poole, UK) by intraperitoneal injection of 10 μg ovalbumin, adsorbed to 1 mg $Al(OH)_3$. From days 14 to 21, the mice were exposed daily to PBS or ovalbumin aerosols (1%) for 30 minutes as previously described.[13]

Airway responsiveness

Airway responsiveness to carbachol was measured 24 hours after the final allergen exposure. The mice were anesthetized with pentobarbital (100 mg/kg intraperitoneally; Sanofi, Libourne, France), and a tracheal cannula was inserted. The femoral artery and the jugular vein were cannulated. The animals were placed on a 37°C heated blanket and ventilated with a Harvard Apparatus mouse ventilator, model 687 (Holliston, Mass) at 130 strokes/min (stroke volume, 0.5 mL). Neuromuscular blockade was induced by injecting pancuronium bromide (1 mg/kg) intravenously (Organon; Teknika N.V., Turnhout, Belgium). Lung resistance was calculated from the differential pressure between the airways and the pleural cavity, tidal volume, and flow. These parameters were measured with a computerized pulmonary mechanics analyzer (Mumed lung function recording system, version 5.0, 1999; Mumed Systems, London, UK). Increasing doses of carbachol were administered intravenously (microinfusion pump: 5, 10, 20, 40, 80 μg/kg). Between each dose, the lung resistance was allowed to return to baseline level. The concentration of carbachol causing a 50% increase of baseline resistance (PC_{50} value) was calculated by log-linear interpolation of the dose-response curve. To evaluate the possible role of the tachykinin NK_1 receptor in airway responsiveness through vagal effects, mice were vagotomized before lung function measurements. Both cervical vagal nerves were dissected free and cut. A piece was resected for histologic evaluation. Control mice were sham-operated. During pilot experiments, heart frequency was evaluated before and after vagotomy. An increased heart frequency was observed in vagotomized animals. The effect of exogenous administered substance P (Bachem, Bubendorf, Switzerland) on carbachol-induced contractions was evaluated (protocol adapted from Folkerts et al[14]). On the basis of the dose-response curve, a dose of carbachol was selected that caused approximately 80% of the maximal response in WT animals. Bronchoconstriction was repeated at 10-minute intervals. The first response was variable, but thereafter, reproducible constrictions could be achieved. After 2 responses to carbachol (80 μg/kg), substance P (40 μg/kg) was injected together with carbachol.

Bronchoalveolar lavage

Immediately after the assessment of airway responsiveness, lungs were lavaged as previously described.[13] A total cell count was performed in a Bürker chamber, and the differential cell counts on at least 400 cells were performed on cytocentrifuged preparations (Cytospin 2; Shandon, Runcorn, UK) by using standard morphologic criteria after staining with May-Grünwald-Giemsa. Cell amounts were expressed as amounts present in the complete lavage sample.

Measurement of ovalbumin specific serum IgE

At the end of the experiment, blood was drawn from the heart for measurement of ovalbumin specific serum IgE. IgE levels were determined as previously described.[13]

Substance P content of bronchoalveolar macrophages

The remaining bronchoalveolar lavage cells (per mouse for ovalbumin-exposed animals; pooled for PBS-exposed or naive animals) were plated in plastic petri dishes pretreated with human and normal goat serum (Gibco BRL, Merelbeke, Belgium) and allowed to adhere for 2 hours at 37°C in a humidified atmosphere containing 5% carbon dioxide. Nonadherent cells were removed by washing monolayers with PBS, and the adherent cells were collected. The macrophages were purified by cell sorting on a FACSvantage flow cytometer (Becton Dickinson, Mountain View, Calif) by using the autofluorescent capacity of these cells. To ensure that the isolated cells were macrophages, cytospin preparations were made and stained with May-Grünwald-Giemsa. The sorted cells were at least 99% macrophages on the basis of morphologic criteria. The macrophages were immediately lysed by resuspending 100,000 cells in 50 μL 2 N acetic acid. The amount of substance P in the lysates was measured in duplicate with the use of a sensitive (20 pg/mL detection limit) competitive peptide enzyme immunoassay (Bachem-Peninsula, San Carlos, Calif) as prescribed by the manufacturer. The assay is specific for substance P with minimal ($<0.01\%$) cross-reactivity for neurokinin A. The concentration of SP in the lysates was used to calculate the amount of SP (pg)/10^6 lysed cells.

Histologic and morphometric analysis

The lungs were infused via the trachea with 4% paraformaldehyde. After excision, the lungs were immersed in fresh fixative overnight. Pieces from all lung lobes were embedded in paraffin and cut in 2-μm–thick sections. Histologic analyses were performed on sections stained with periodic acid-Schiff, Congo red (0.5% in 50% ethanol), or eosin/hematoxylin, respectively. In the stained tissue sections, airways cut in a reasonable cross-section (defined by a ratio of maximal internal diameter to minimal internal diameter <1.8) and with a length of basement membrane (Pbm) >800 μm were examined by light microscopy at magnification 200×. A camera sampled the image of each airway and quantitative measurements were performed on the digital representation of the airways via a computerized image analysis system (KS400; Zeiss, Oberkochen, Germany). The morphometrical parameters[15] that were marked manually on the digital representation of the airway were as follows: Pbm, the area defined by the basement membrane (Abm), and the area defined by the total adventitial perimeter (Ao). The total bronchial wall area (WAt) was calculated from these values (WAt = Ao − Abm), and WAt was normalized to the square length of the basement membrane

(WAt/Pbm^2). Airway morphometry was measured in hematoxylin and eosin stained sections. Goblet cells were quantified in periodic acid-Schiff stained sections. Results were expressed as number of goblet cells per millimeter basement membrane. Peribronchial infiltration with eosinophils was evaluated in lung sections stained with Congo red and expressed as total number of eosinophils per square millimeter WAt.

Statistical analysis

All results are reported as means ±SEMs. Dose-response curves were compared through univariate ANOVA. Post hoc analysis (least significant difference or Scheffé) for groups was performed when ANOVA test was significant. Bronchoconstriction in the presence or absence of substance P was compared with a paired samples *t* test. Data of quantitative measurements in the airway wall of different mice were pooled together. Mean values of different groups were compared through the Kruskal-Wallis test for multiple comparisons. If significance was reached between the groups, pairwise comparisons were made by using a Mann Whitney *U* test with Bonferroni corrections. Differences were regarded as significant when *P* values were $<.05$. The statistical analyses were accomplished with SPSS for Windows 11.0 software (SPSS Inc, Chicago, Ill).

RESULTS

BAL fluid

Sensitization and subsequent exposure to ovalbumin induced a significant increase in total cell numbers in BAL fluid ($P<.01$; Table I). No differences could be observed between ovalbumin-exposed WT (n = 11) and tachykinin NK_1 receptor knockout animals (n = 10). Differential cell counts showed that ovalbumin-exposed groups also had significant increases in percentages of eosinophils ($P<.001$), macrophages ($P<.001$), and lymphocytes and neutrophils ($P<.05$) in their BAL fluid compared with PBS-exposed controls (WT, n = 11; knockout, n = 9; Table I). No differences in eosinophil, macrophage, lymphocyte, and neutrophil numbers were observed between ovalbumin-exposed tachykinin NK_1 receptor knockout and ovalbumin-exposed WT mice. A more detailed figure is provided in the Journal's Online Repository (Fig E1; see www.mosby.com/jaci). A possible trend toward a lower number of cells in the knockout mice was not confirmed in additional, independent experiments. Total cell numbers in lavage fluid from naive animals did not differ from each other. Differential cell counts revealed that lavage samples from untreated mice contained nearly 100% macrophages (Table I).

Peribronchial eosinophils

Both tachykinin NK_1 receptor WT and knockout animals developed peribronchial eosinophilia on sensitization and exposure to ovalbumin ($P<.001$; Table II). The numbers of infiltrated eosinophils per square millimeter of airway wall were similar in ovalbumin-exposed tachykinin NK_1 receptor WT and ovalbumin-exposed tachykinin NK_1 receptor knockout mice.

Ovalbumin specific IgE

As proof of active immunization, ovalbumin-specific serum IgE levels were elevated in sensitized mice exposed to ovalbumin compared with those in PBS-exposed animals ($P<.001$ in WT; $P<.01$ in knockout; Table II). No significant difference could be observed between ovalbumin-exposed tachykinin NK_1 receptor WT and ovalbumin-exposed tachykinin NK_1 receptor knockout mice.

Goblet cells

Ovalbumin exposure induced goblet cell hyperplasia in both tachykinin NK_1 receptor WT and knockout animals ($P<.001$ vs PBS-exposed controls; Table II). The amount of goblet cells per millimeter of basement membrane (Pbm) was significantly higher in the ovalbumin-exposed tachykinin NK_1 receptor WT mice ($P<.01$ vs ovalbumin-exposed knockout mice; Table II).

Substance P content

No differences in substance P content of bronchoalveolar macrophages could be observed between naive tachykinin NK_1 receptor WT and knockout animals (Table III). The influence of ovalbumin sensitization and exposure was also evaluated. No differences could be observed between PBS and ovalbumin-exposed groups or between ovalbumin-exposed tachykinin NK_1 receptor WT and ovalbumin-exposed tachykinin NK_1 receptor knockout mice (Table III).

Morphometry

No differences in airway wall thickness could be observed between naive tachykinin NK_1 receptor WT and knockout animals (Table III). A 1-week allergen exposure did not induce an increase of the airway wall thickness of tachykinin NK_1 receptor WT and knockout animals. No differences could be observed between PBS-exposed and ovalbumin-exposed groups or between ovalbumin-exposed tachykinin NK_1 receptor WT and ovalbumin-exposed tachykinin NK_1 receptor knockout mice (Table III).

Airway responsiveness

Fig 1, *A*, shows the dose-response curve of the *in vivo* airway responsiveness to intravenous carbachol of naive tachykinin NK_1 receptor knockout and WT animals. Tachykinin NK_1 receptor knockout mice responded significantly more to carbachol than the tachykinin NK_1 receptor WT mice, as demonstrated by a significant leftward shift of the curve and a decreased PC_{50} value ($P<.001$). Vagotomy did not influence the response to carbachol in the naive tachykinin NK_1 receptor WT or in the naive tachykinin NK_1 receptor knockout mice (Fig 1, *B*). No shift of the dose-response curve to carbachol or a decrease in PC_{50} value was observed. Vagotomized tachykinin NK_1 receptor knockout mice were still more responsive than the WT counterparts. Baseline lung resistance was the same for tachykinin NK_1 receptor knockout and WT animals. Exogenous administered

TABLE I. Total and differential cell count in BAL fluid

Group	Total cells ($\times 10^3$)	Macrophages (%)	Eosinophils (%)	Lymphocytes (%)	Neutrophils (%)
WT-naive	91.4±21.1	97.47±0.39	0.1±0.07	1.71±0.51	0.71±0.35
KO-naive	147.5±20.1	97.80±0.62	0.38±0.27	1.58±0.65	0.24±0.07
WT-PBS	190.0±28.5	98.27±1.74	0.31±0.31	1.05±0.29	0.35±0.15
WT-OVA	2492.7±938.6†	50.11±6.90‡	46.1±7.26‡	2.03±0.82	1.82±0.50
KO-PBS	136.3±15.5	98.8±0.33	0.10±0.10	0.93±0.19	0.18±0.10
KO-OVA	767.0±258.4†	58.47±7.87‡	36.00±7.84‡	5.01±1.12*	0.54±0.24

KO, NK_1 receptor knockout; *OVA,* ovalbumin.
*$P < .05$.
†$P < .01$.
‡$P < .001$, PBS vs OVA.

TABLE II. Ovalbumin specific serum IgE, peribronchial eosinophils, and goblet cells in epithelium

Group	IgE(U/mL)	Eosinophils (cells/mm^2 airway wall)	Goblet cells (cells/mm Pbm)
WT-PBS	1.95±0.25	41.40±5.01	0.05±0.04
WT-OVA	63.14±12.15†	211.88±25.22†	40.27±4.93†
KO-PBS	1.70±0.49	40.73±5.63	0.03±0.02
KO-OVA	38.16±6.83*	176.50±26.79†	21.76±4.08† ‡

KO, NK_1 receptor knockout; *OVA,* ovalbumin.
*$P < .01$.
†$P < .001$, PBS vs OVA.
‡$P < .01$, WT-OVA vs KO-OVA.

TABLE III. Airway wall thickness and substance P content in alveolar macrophages

	Airway wall thickness (WAt/Pbm^2)		Substance P content (pg/100,000 cells)	
Treatment	WT	Knockout	WT	Knockout
Naive	23.2±0.9	24.2±0.8	402±14	405±14
PBS exposure	19.7±0.8	17.7±0.7	462±58	487±26
Ovalbumin exposure	21.4±0.9	21.1±1.4	440±20	450±21

substance P together with carbachol decreased the observed bronchoconstriction to carbachol alone in the tachykinin NK_1 receptor WT mice (57.28% ± 13.29% vs 82.24% ± 15.96% increase in lung resistance; n = 8; $P < .01$). This inhibitory effect was not observed in the tachykinin NK_1 receptor knockout animals (129.88% ± 25.34% vs 134.60% ± 27.79% increase in lung resistance; n = 8). A 1-week exposure to aerosolized ovalbumin was not able to induce airway hyperresponsiveness to carbachol in the tachykinin NK_1 receptor WT and the knockout mice versus the PBS-exposed controls. Neither leftward shift of the dose-response curve nor a decrease in PC_{50} value could be observed in either strain ($P > .05$; Fig 1, *C*). However, PBS-exposed and ovalbumin-exposed tachykinin NK_1 receptor knockout mice respond significantly more to carbachol in comparison with the WT counterparts. This is illustrated by a significant leftward shift of the dose-response curve ($P < .01$) to carbachol in the PBS-exposed animals and in the ovalbumin-exposed mice.

DISCUSSION

In our mouse model, no effect of the tachykinin NK_1 receptor on the allergic airway inflammation could be observed. Ovalbumin-induced inflammatory cell influxes in BAL fluid and ovalbumin-specific serum IgE levels of tachykinin NK_1 receptor knockout mice were similar to those obtained from WT littermates. Also, the peribronchiolar eosinophilia was similar in both groups, suggesting no proinflammatory action of tachykinins through this receptor. These findings corroborate studies in rats and guinea pigs in which the use of tachykinin NK_1 receptor antagonists indicated that the tachykinin NK_1 receptor is not involved in antigen-induced inflammatory cell influxes in BAL.[9-11] Our results contrast with another study in guinea pigs in which signaling through the tachykinin NK_1 receptor augmented the allergen-induced infiltration of eosinophils, neutrophils, and lymphocytes in the airways.[3] Differences according to species specificity and/or methodology may explain these conflicting results. In another mouse model of lung inflammation, a NK_1 receptor antagonist reduced significantly the total number of inflammatory cells retrieved by BAL,[16] but this model represented inflammation compartmentalized in lung parenchyma. Several studies using tachykinin NK_1 receptor knockout mice suggested a role for this receptor in inflammation in various organs.[1] Bozic et al[17] reported that the disruption of the tachykinin NK_1 receptor protected the lung from immune complex injury. Their animal model is also not designed to evaluate allergic airway inflammation and therefore does not contrast with our observations.

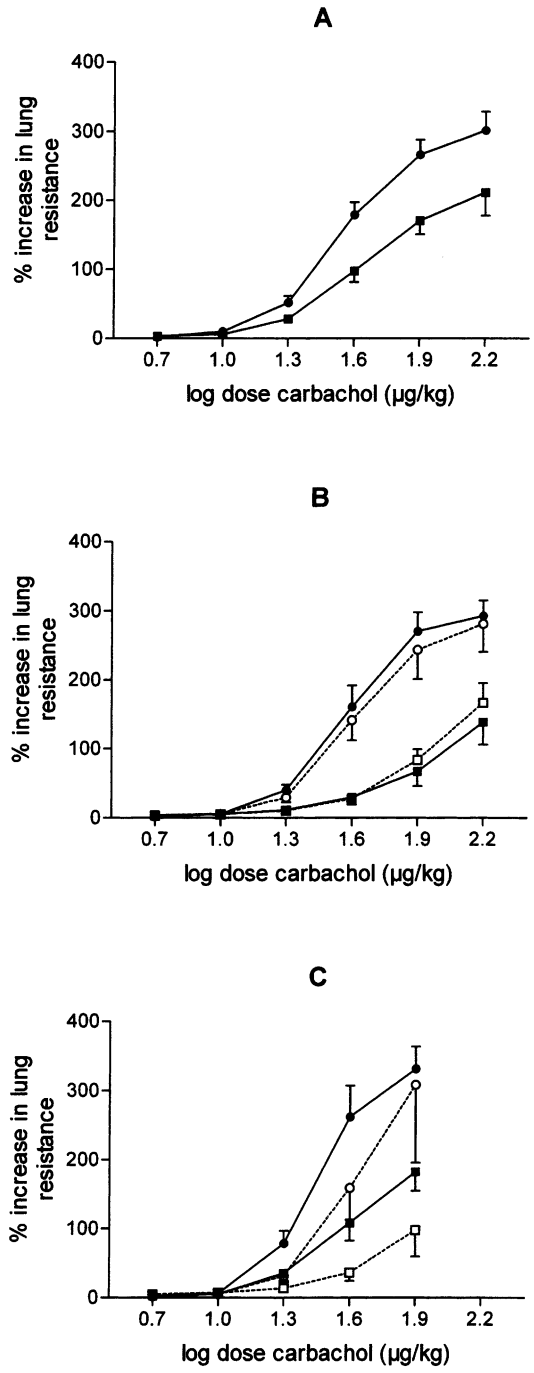

FIG 1. A, Airway responsiveness to carbachol in naive NK_1 receptor WT *(closed squares)* and NK_1 receptor knockout animals *(closed circles)*. **B,** Responsiveness to carbachol in vagotomized and sham-operated naive WT (*open* and *closed squares,* respectively) and naive knockout animals (*open* and *closed circles*). **C,** Responsiveness to carbachol in ovalbumin-exposed and PBS-exposed WT (*open* and *closed squares*) and knockout (*open* and *closed circles*) animals.

Because macrophages are the first line of defense, they may play a role in allergic inflammation. An autocrine and autofeedback function (maintaining baseline and increasing endogenous substance P levels on stress) for substance P in these cells has been suggested.[8,18-20] Pascual and Bost[8] reported that P388D1 macrophages produced substance P and responded to that production in an autocrine or paracrine fashion, leading to release of IL-1–like activity during stress. Lai et al[18] demonstrated that a specific NK_1 receptor antagonist abrogated not only the exogenous substance P–induced substance P mRNA expression but also resulted in substance P mRNA expression that was lower than that of baseline control, suggesting that endogenously produced substance P controlled its own mRNA expression. Therefore, we hypothesized that the lack of the receptor might influence the endogenous substance P levels of alveolar macrophages. However, no differences in endogenous substance P levels of these cells from NK_1 receptor WT and knockout animals were observed, suggesting no autofeedback mechanism in these conditions. Furthermore, if substance P and its receptor were involved in an autocrine loop to release proinflammatory cytokines on inflammation, as suggested by Pascual and Bost, [8] we expected to observe higher substance P levels in alveolar macrophages of allergic WT animals compared with controls, because signaling through the receptor would also activate the autofeedback system. Although higher numbers of alveolar macrophages were retrieved from lungs of ovalbumin-treated animals, no alterations of endogenous substance P levels from macrophages from PBS-exposed and ovalbumin-exposed WT mice were observed. These data suggest that the endogenous substance P levels are not upregulated and probably not involved in the augmentation of cytokine release in this model of allergic inflammation. However, the activation status of the macrophages of ovalbumin-exposed WT and knockout animals may be different, as was suggested by an *in vitro* study in which release of endogenous substance P altered the activation status of P388D1 macrophages.[8] If this is the case, nevertheless, no differences in inflammation are induced by the altered activation status. Furthermore, antagonizing the NK_1 receptor has no influence on the increased activation status of alveolar macrophages after ovalbumin challenge in sensitized guinea pigs, suggesting no effect of the NK_1 receptor on the activation of macrophages in ovalbumin-sensitized and ovalbumin-exposed animals.[11]

We did not observe any induced hyperresponsiveness in our model, although others did report allergen-induced hyperresponsiveness in mice,[21,22] guinea pigs,[23,24] and rats.[25] Therefore, no conclusions about the role of the NK_1 receptor in allergen-induced hyperresponsiveness could be made. This conflicting result may be related to the marked variation among the used models with respect to the background strains of the animals,[26-28] the variety of sensitization and challenge protocols used, and the different readouts used to assess the airway responsiveness.[29,30] It is of interest to note that in naive animals lacking the tachykinin NK_1 receptor, an increased responsiveness to intravenous carbachol was observed. This observation corroborates the fact that mice with a targeted deletion of the tachykinin I gene (mice lacking

substance P) are hyperresponsive to intravenous methacholine.[31] Hyperresponsiveness must be a function of excessive airway narrowing. Possible mechanisms responsible for this excessive narrowing include altered neural pathways, remodeling of the airways, and the presence of inflammatory mediators.[32] In this particular case, the effect of inflammatory mediators can be neglected, because in naive animals, no inflammation was observed. Moreover, no altered airway morphology was observed. The thickness of the airway wall was similar in tachykinin NK_1 receptor WT and knockout animals. The dominant neural control of the airway smooth muscle is provided by the parasympathetic fibers of the vagus nerves. The tachykinin NK_1 receptor may be involved in central vagal control.[10,33] However, cutting of these vagus nerves had no effect on the observed responsiveness in the naive NK_1 receptor knockout animals or in the WT mice. This means that central neural elements do not influence the observed response to carbachol. Altered smooth muscle contractility to carbachol in the NK_1 receptor knockout animals does not seem to be the cause, either.[34] On sensory nerve activation (through contraction), an antidromic release of tachykinins from c-fibers via an axon reflex may occur. These peptides may in turn mediate a relaxation through binding with the epithelium and subsequent release of prostaglandin, as was described *in vitro*.[34] Exogenous substance P indeed has an inhibitory effect on carbachol-induced bronchoconstriction in NK_1 receptor WT mice. This inhibition was abolished in the NK_1 receptor knockout mice, suggesting that this protective effect is mediated through the NK_1 receptor. Exposure to ovalbumin increased the numbers of goblet cells in both WT and knockout animals, but the amount of goblet cells per millimeter of basement membrane was significantly less in the knockout mice, suggesting a role for the NK_1 receptor in goblet cell hyperplasia. Increased numbers of goblet cells are considered part of airway remodeling in asthma.[35] The hyperplasia is linked with increased mucus secretion and consequent airflow obstruction, an important feature of asthma. The mechanisms underlying the development of goblet cell hyperplasia are now being unraveled, mainly through the use of murine asthma models. Several mechanisms may explain our findings. First, inhaled allergens induce a T_H2 lymphocyte response with release of cytokines that induce goblet cell hyperplasia either directly or indirectly.[36] As substance P[37] and its receptor[38,39] are expressed in and on lymphocytes, these cells may be influenced in an autocrine or paracrine fashion to augment the release of these cytokines. Several studies have already shown that substance P is able to modulate the chemotaxis, proliferation, and activation of lymphocytes.[40] Second, substance P may influence the goblet cell hyperplasia by a direct effect on tachykinin NK_1 receptors on the epithelium. The peptide may be released by a variety of stimuli and stimulate goblet cells to proliferate. Substance P is present in nerve profiles, found beneath and within the epithelium and around submucosal glands.[41,42] Furthermore, Chu et al[43] demonstrated that epithelial cells are an additional source of substance P. They also demonstrated expression of the tachykinin NK_1 receptor on goblet cells. Further evidence for this hypothesis is provided by their work demonstrating increased expression of substance P and its receptor in asthmatics, which was correlated with the mucus content in the airway epithelium.[43] In conclusion, the tachykinin NK_1 receptor is involved in the secretory response of goblet cells,[44-48] and we provide evidence for an involvement of this receptor in proliferation of these cells induced by antigen.

To conclude, we can state that despite the fact that the tachykinin NK_1 receptor is not involved in the antigen-induced airway inflammation, it does augment the antigen-induced goblet cell hyperplasia in a mouse model of allergic asthma. This may have important implications for the use of specific NK_1 receptor antagonists in pathologies like asthma, in which goblet cell hyperplasia and mucus hypersecretion are important features.

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