

ORIGINAL ARTICLE

Nerve growth factor mediated SH2-B β /Akt signal pathway activated in allergic airway challenge in mice

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ABSTRACT

Background and objective: Nerve growth factor (NGF) contributes to airway inflammation and bronchoconstriction in allergic asthma. The Src homology 2 β /serine/threonine kinase (SH2-B β /Akt) pathway is one of the avenues through which NGF regulates the biological activity of pheochromocytoma (PC)12 cells. It has also been reported that NGF upregulates the expression of SH2-B β in the lung tissue of asthmatic mice. The present study investigated the effects of NGF and SH2-B β on Akt activation during allergic airway challenge.

Methods: BALB/c mice were sensitized and challenged with ovalbumin. The effects of NGF and SH2-B β on Akt in allergic airway challenge were assessed by intravenously administering anti-NGF antibody or a mutant of SH2-B β (R555E) to these mice. Pulmonary histological changes were then assessed and the inflammatory cells in the BAL fluid (BALF) were counted. Additionally, phosphorylated Akt (p-Akt) expression was determined by fluorescence microscopy, western blotting and quantitative RT-PCR. Airway resistance was also measured using closed-type body plethysmography.

Results: We observed p-Akt overexpression in the lungs after allergen challenge by fluorescence microscopy, Western blotting and RT-PCR, as compared with the control. However, after treatment with anti-NGF or R555E, p-Akt levels and allergen-induced airway inflammation were reduced in comparison with those of allergen-challenged mice. Anti-NGF and R555E also decreased airway hyperresponsiveness caused by allergen challenge in response to methacholine (MCH).

Conclusions: These results suggest that SH2-B β regulation of Akt partly participates in the NGF-mediated development of allergic airway challenge.

SUMMARY AT A GLANCE

The study investigated the effects of NGF and SH2-B β on Akt activation during allergic airway challenge using BALB/c mice sensitized with ovalbumin. The results indicate that the SH2-B β regulation of Akt participates in the NGF-mediated allergic airway response and blocking NGF and SH2-B β may be a potential therapeutic approach for asthma.

Key words: allergy, Bagb albino/c mouse, nerve growth factor, serine/threonine kinase, Src homology 2 β .

INTRODUCTION

Asthma and several other respiratory diseases are characterized by airway inflammation. Many cell types and cytokines contribute to this inflammation, which enhances bronchial sensitivity to a variety of stimuli.¹ Nerve growth factor (NGF) has been specifically implicated in the pathogenesis of asthma due to its ability to promote sensory neuron hyperreactivity and induce airway inflammation.^{2,3} In addition, NGF regulates pheochromocytoma (PC)12 cells, in part by stimulating phosphorylation of Src homology 2 β (SH2-B β) on serine/threonine kinase (Akt/PKB).⁴⁻⁷ SH2-B β is a member of a widely expressed, highly conserved family of adaptor proteins and can bind to Janus kinase 2⁸ and the activated receptor tyrosine kinases for insulin, insulin-like growth factor I, NGF, platelet-derived growth factor, and fibroblast growth factor.⁹⁻¹³ Moreover, SH2-B β is critical for both NGF-dependent neurite outgrowth and neurite maintenance.¹⁴ Additionally, Wang *et al.* has described SH2-B β enhancement and prolongation of NGF-induced Akt phosphorylation and of Akt enzymatic activity in PC12 cells.¹⁵ Li *et al.* has also characterized SH2-B β expression in alveolar macrophages in the BAL fluid (BALF) of asthmatic guinea pigs and its role

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in NGF tyrosine kinase receptor A (TrkA)-mediated asthma.¹⁶ Together, these studies suggest that SH2-B β plays a fundamental role in receptor tyrosine kinase-mediated cellular functions. Based on the above information, we hypothesized that upregulation of Akt phosphorylation by SH2-B β may contribute to the pathogenesis of NGF-mediated asthma. The aim of the current study was to investigate Akt activation as well as the regulation of Akt signal protein by NGF and SH2-B β after allergic airway challenge in ovalbumin (OVA)-sensitized BALB/c mice.

METHODS

Animals

Fifty-five normal female BALB/c mice (6–8 weeks old) weighing 22 ± 2.5 g were obtained from the Laboratory Animal Research Center in Beijing, China. These mice were maintained with 12 h/12 h light/dark cycles and free access to food and water. At the beginning of the study, they were randomly divided into four groups: control ($n = 12$), ovalbumin (OVA, $n = 16$), R555E ($n = 12$) and anti-NGF ($n = 15$). The average respiratory rate of each group was 108 ± 9 , 107 ± 12 , 108 ± 12 and 107 ± 10 times/min, respectively, with no significant differences between groups ($P > 0.05$). All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Additionally, the institutional ethics committee for animal use and care and the internal review board approved all animal-related experiments and procedures.

OVA sensitization and aerosol exposure, anti-NGF and R555E treatment

Sensitization and challenge protocols were performed according to Elwood *et al.*¹⁷ and Vanacker *et al.*¹⁸ with some modifications, as described below.

On days 0, 7, 14 and 21, all mice except the control group were actively sensitized with an intra-abdominal injection of 1 mg OVA (Sigma, St Louis, MO, USA) and 100 mg aluminum hydroxide in 0.5 mL PBS; control group were injected with 0.5 mL PBS only. The control group mice were then exposed to aerosolized PBS for 30 min/day from day 22 to day 30. Meanwhile, the OVA-sensitized mice were exposed to 10 mg/mL aerosolized OVA (1 g OVA in 100 mL sterile PBS in a nebulizer) for 30 min/day from day 22 to day 33. The anti-NGF group and R555E group mice were anesthetized and administered an intravenous injection of 50 μ L polyclonal rabbit anti-mouse NGF antibody (Sigma, St Louis, MO, USA) diluted 1:10 in PBS and 50 μ L R555E. This SH2-B β mutant was generated using the QuickChange site-directed mutagenesis method (Stratagene, Foster City, CA, USA) to full-length SH2-B β cloned by RT-PCR from a rat-brain cDNA library. Intravenous treatment was administered 3 h before allergen aerosol challenge on days 31, 32 and 33.

One day after the last of the OVA exposures, mice were sacrificed and their lung tissues were collected and divided into two parts. One part was promptly frozen at -80°C for western blotting and RT-PCR analysis, while the other was immediately fixed in 4% paraformaldehyde for HE staining and immunofluorescence microscopy.

HE staining

Lungs were fixed in 4% formaldehyde in PBS and 5- μ m slices were cut and stained with HE according to standard protocols. The lung tissue slices were then examined using a light microscope (Olympus, Tokyo, Japan) by an observer blinded to the experimental groups. The tissue was scored for inflammatory cells, mucosal oedema and epithelial lesions based on the following scale: absent (0), rare (1), mild (2), moderate (3) or severe (4).

Determination of airway hypersensitivity

Twenty-four hours after the final challenge, mice were anaesthetized and the trachea was cannulated and airway resistance was measured using AniRes 2005 animal lung function analysis (Peking Biolab Tech Co., Beijing, China). As described by Xu *et al.*¹⁹ and Zhu *et al.*²⁰ the mice were anaesthetized, a tracheotomy was performed, and a cannula connected to a breathing machine and a signal regulation system was inserted. The mice were then placed in a sealed whole-body plethysmograph and connected to a rodent ventilator at a breath frequency of 90 times per minute. Transducers (ML-AMP II, Peking SYNOL High-Tech Co., Beijing, China) were connected to the ventilatory circuit with set voltage signals for pressure and flow, which were amplified and recorded for later analysis. A microcomputer running the AniRes2005 software was used to continuously calculate the inspiratory resistance (IR) and expiratory resistance (ER) of the respiratory system from the digitized pressure and flow signals. After stabilization of the respiratory parameters, the mice were given intravenous methacholine (MCH, Sigma, dissolved in 0.9% sodium chloride) in gradient doses (0.025, 0.05, 0.075, 0.1 mg/kg) via a 22 G catheter inserted into the external jugular vein. Injections were administered at 5 min intervals. The MCH volume was 50 μ L, delivered over 3–4 s, based on the criterion that the resistance curves must return to the pre-MCH level before the next MCH injection. The response was measured as the peak increase above the baseline immediately after MCH administration.

BALF sample collection

BALF samples were collected 24 h after the last challenge. After killing the mouse by cervical dislocation, the lungs were lavaged with 1.6 mL (0.8 mL per lavage) ice-cold PBS via the tracheal cannula. The lavage fluid was recovered by gentle manual

aspiration with a syringe. The retrieved volume, averaging 75–80% of the instilled PBS, was then centrifuged at 500 g. After discarding the supernatant, the resultant pellet was washed twice with PBS and resuspended in 1 mL PBS. Total BALF cell counts were performed using a haemocytometer. A differential cell count was carried out by Wright-Giemsa staining on the basis of morphologic criteria, using a light microscope to evaluate at least 200 cells per slide. All slides were counted twice by different observers blinded to the animal's previous treatment.

Immunolocalization of p-Akt

Prior to immunofluorescent staining for pulmonary phosphorylated Akt (p-Akt), the excised lung tissue was infused with 4% paraformaldehyde and post-fixed in sucrose for 24 h. The lungs were then immersed in 30% sucrose in PBS for an additional 24 h, frozen in liquid nitrogen, and stored at -80°C . Frozen sections were blocked with rabbit serum for 30 min at room temperature and then incubated with 1:200 mouse polyclonal anti-p-Akt antibody (Cell Signaling Technology, Inc., Beverly, MA, USA) at 37°C for 2 h. After washing twice with PBS, the sections were incubated with 1:100 anti-rabbit IgG conjugated to fluorescein isothyanate (Sigma, St Louis, MO, USA) for 30 min at room temperature, shielded from light. The sections were then mounted with glycerol and images were captured using an Olympus BX 60 Upright Fluorescence microscope.

Western blot analysis

Protein homogenates of lung tissue samples were prepared by rapid homogenization in 10 volumes of lysis buffer (2 mM EDTA, 10 mM EGTA, 0.4% NaF, 20 mM Tris-HCl, 1 $\mu\text{g}/\text{mL}$ leupeptin, 1 $\mu\text{g}/\text{mL}$ aprotinin, and 1 mM Na_3VO_4 pH 7.5). Samples were centrifuged at 17 000 g for 1 h and the protein concentration of the soluble material was determined using the Coomassie G250 binding method. Ten micrograms of protein from each sample was then loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel. Electrophoreted proteins were transferred from the gel to nitrocellulose membranes, which were incubated with 1:1000 rabbit polyclonal anti-p-Akt. The p-Akt band (60 kDa) was visualized using an enhanced chemiluminescence kit (Santa Cruz, CA, USA). Integrated density values (IDV) were analysed using a computerized image analysis system (Fluor Chen 2.0) and normalized to those of β -actin.

RNA isolation and RT-PCR amplification

RT-PCR was performed using the TaKaRa RNA PCR Kit (AMV) Ver.3.0 (Takara Biotechnology, Dalian, China). Total RNA was extracted from the lung tissue and purified using RNAout (Takara Biotechnology)

according to the manufacturer's instructions. The primers for the Akt subunit were 5'-ATGCTGGAC AAGGACGGG-3' (forward) and 5'-CACGATGTTGGC AAAGAA-3' (reverse) and those for β -actin were 5'-GTGGGGCCCCAG GCACCA-3' (forward) and 5'-GCTCGGCCGTGGTGGTGAAGC-3' (reverse). The reaction was carried out for 30 cycles, using a 94°C , 30 s denaturing step; a 52°C , 40 s annealing step; and a 72°C , 1 min extension step. The procedures conformed to a standard protocol. For semi-quantitative measurement of Akt density, the involved slides were photographed and assessed using computer-aided image analysis (Motic Images Advanced 3.2, Motic, Xiamen, China).

Data analysis

All values are expressed as the mean $\pm$ SD. One-way analysis of variance (ANOVA) followed by the Tukey test was used to compare group differences in Akt levels, while two-way ANOVA was used to assess differences in airway resistance. A P -value of <0.05 was considered statistically significant.

RESULTS

Pathological changes in the bronchi and lung tissue

Histological scoring of tissue sections obtained from the experimental mice confirmed pulmonary histopathological changes (Fig. 1a). Inflammatory cells, mucosal oedema and epithelial lesions were significantly more prevalent in untreated asthmatic mice than in those treated with anti-NGF or R555E ($P < 0.01$). The total number of cells in the BALF was significantly higher in OVA-sensitized mice than in the control mice (Fig. 1b). The number of macrophages, eosinophils and lymphocytes was also significantly increased in OVA-sensitized mice but reduced in anti-NGF- and R555E-treated mice (Fig. 1c). Moreover, many inflammatory cells, including mononuclear cells, macrophages, lymphocytes and eosinophils, were observed in the asthmatic pulmonary alveoli and surrounding bronchi by HE staining (Fig. 2a-B). The airway mucosa of the OVA-sensitized mice also exhibited obvious oedema with bronchiolar wall thickening, causing stenosis of the lumen and the alveolar interstitium, while the pulmonary tissue showed smooth muscle hyperplasia, in contrast with the control mice (Fig. 2a-A). However, fewer infiltrating inflammatory cells were seen in the peribronchial and perialveolar areas of anti-NGF- and R555E-treated mice (Fig. 2a-C,D, respectively).

Airway resistance measurement

All experimental mice exhibited dose-dependent augmentation of IR and ER in response to increasing

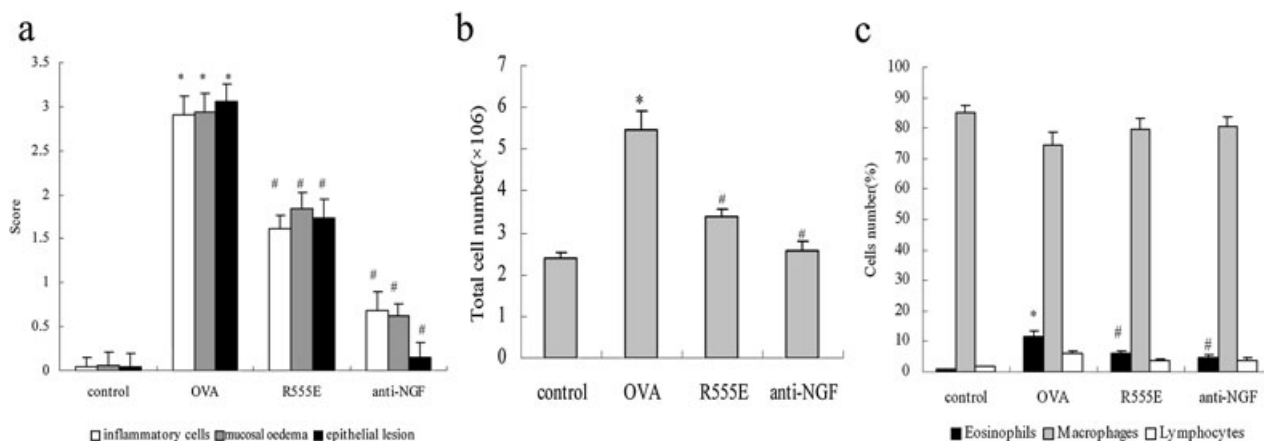


Figure 1 (a) Histological scoring of sections confirmed the presence of different histopathological changes in the lung. Inflammatory cells (□), mucous oedema (▨) and epithelial lesions (■) were significantly more prevalent in ovalbumin (OVA)-sensitized mice than in control mice but reduced in anti-NGF- and R555E-treated mice ($P < 0.01$). (b) The total and (c) differential cell numbers in the BAL fluid were also determined. The total cell numbers and macrophages (▨), lymphocyte (□) and eosinophil (■) counts in BALF were significantly higher in the OVA-sensitized mice than in control mice but reduced in anti-NGF- and R555E-treated mice ($P < 0.01$). vs. control group * $P < 0.01$, vs. OVA group # $P < 0.01$. NGF, nerve growth factor.

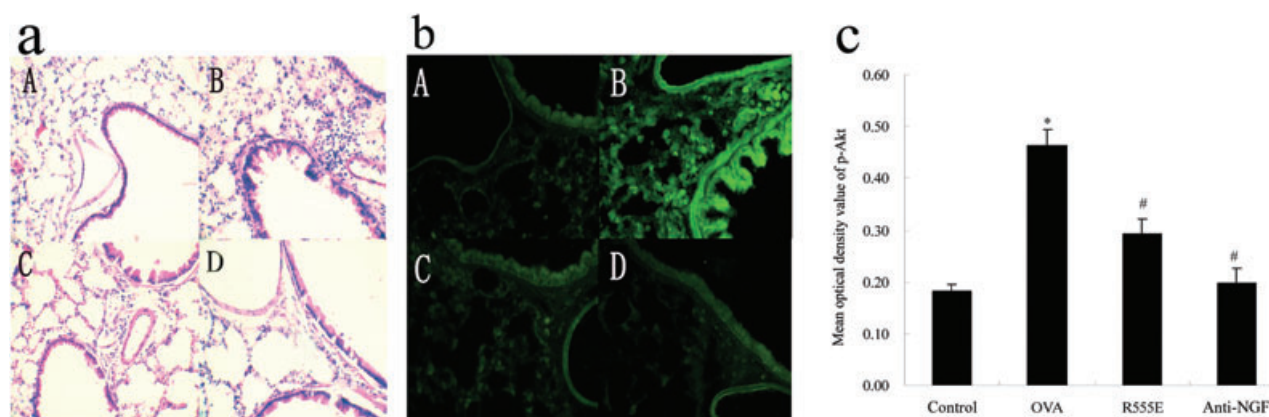


Figure 2 (a) Pathological changes in the bronchi and lung tissue, as detected by HE staining at ×400. The surrounding bronchi and pulmonary alveoli show obvious inflammatory cell infiltration, and obvious airway mucosa oedema with airway smooth muscle hyperplasia can be seen in the ovalbumin (OVA) group. (C, D) The above changes significantly relieved after intervention with the anti-NGF and R555E. (b) Immunofluorescence at ×400 showed expression and localization of p-AKT in the lung tissue of the (A) control group, (B) OVA group, (C) R555E group and (D) anti-NGF group. The green fluorescence emitted by p-Akt can be seen in the lung tissue, and the fluorescence from OVA group was obviously more intense than those from the control group, the anti-NGF group or R555E group, indicating activation of the p-Akt in the OVA group and NGF and SH2-B β upregulating expression of p-Akt. (c) Mean optical density values (mean $\pm$ SD) in lung tissues by immunohistochemistry detection. Versus control group * $P < 0.01$, versus OVA group # $P < 0.01$. NGF, nerve growth factor.

doses of intravenously administered MCH. There was no statistically significant difference in baseline IR and ER values among all experimental groups. The airway response to MCH was increased in the OVA group compared with the control group ($P < 0.01$). Treatment of OVA-sensitized mice with intravenous anti-NGF and R555E prior to OVA challenge prevented this increase in airway reactivity, reflected by a shift in the dose-response curves (Fig. 3a,b) and significantly decreased reactivity compared with the untreated OVA-sensitized mice ($P < 0.01$).

Immunofluorescent analysis of the effects of NGF and SH2-B β on Akt activation

Immunofluorescent staining of lung tissue from the OVA group showed that p-Akt levels were significantly increased in the macrophages, eosinophils and lymphocytes in the pulmonary alveoli, the surrounding bronchi, the small airways, bronchi-associated blood vessels, airway smooth muscle and tracheal epithelium (Fig. 2b-B) ($P < 0.01$), as compared with the control group (Fig. 2b-A). The overexpression of p-Akt

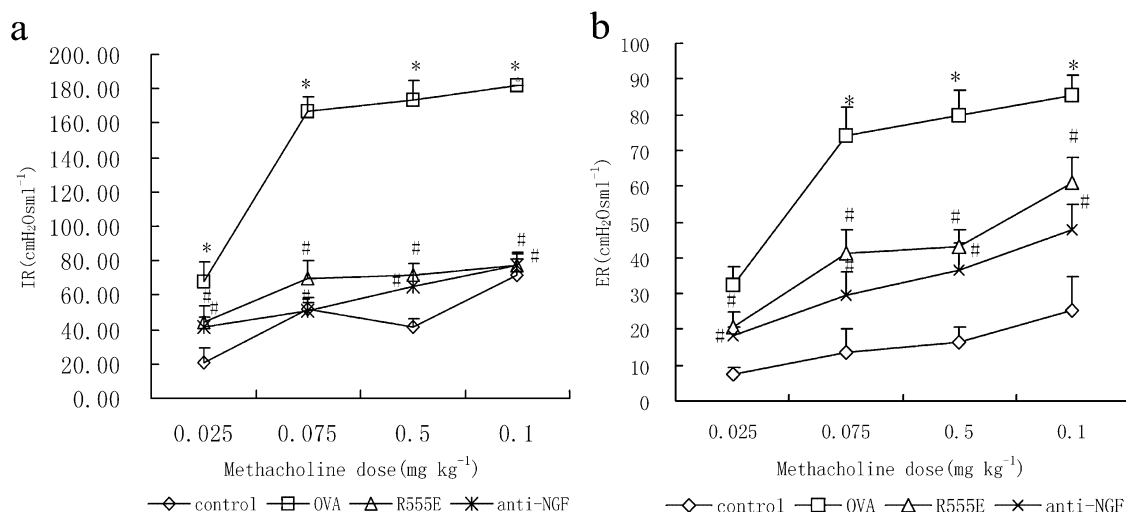


Figure 3 (a) Airway inspiratory resistance (IR) and (b) airway expiratory resistance (ER) of four groups with regard to MCH dose-response were analysed using an animal lung function apparatus. The dose-response curves reflected that the airway response to MCH was increased in the (□) OVA group compared with the (◇) control group ($P < 0.01$). Treatment of OVA-sensitized mice with (✱) anti-NGF and (△) R555E prevented this increase in airway reactivity ($P < 0.01$). Versus control group ** $P < 0.05$ or * $P < 0.01$, versus OVA group # $P < 0.01$ or ## $P < 0.05$.

at the above sites was reduced in both the anti-NGF- and R555E-treated groups (Fig. 2b-C,D, respectively). Immunohistochemistry confirmed the mean optical density values of p-Akt in lung tissues of the four groups, as shown in Figure 2c.

Western blot analysis revealed p-Akt protein expression

p-Akt protein levels in all groups were detected by western blotting. In the lung tissue of the control group, p-Akt was expressed at low levels, but these levels were markedly increased in the OVA group. More specifically, the IDV ratio of p-Akt to β -actin was twofold higher in the OVA group than in the control group. However, anti-NGF and R555E greatly decreased the p-Akt upregulation caused by OVA (Fig. 4a). The IDV ratios of p-Akt to β -actin were 0.188 ± 0.027 in the control group, 0.357 ± 0.021 in the OVA group, 0.0195 ± 0.017 in the anti-NGF group and 0.0256 ± 0.031 in the R555E group (Fig. 4b).

Effects of anti-NGF and R555E on Akt mRNA

RT-PCR was used to examine the expression levels of Akt mRNA in the pulmonary tissue of all groups. Akt mRNA expression was significantly higher in the OVA group than in the control group, but anti-NGF and R555E treatment significantly decreased Akt mRNA expression in these OVA-sensitized mice (Fig. 4c). The IDV of p-Akt mRNA in the lungs of the four groups are shown in Figure 4d.

DISCUSSION

This study investigated Akt activation and the regulatory effects of NGF and SH2-B β on Akt using an

OVA-induced, allergic airway inflammation BALB/c model. We found that Akt was activated during the development of allergic airway inflammation and the intravenous administration of anti-NGF or a mutant of SH2-B β could downregulate this activation and inhibit the NGF-mediated inflammatory response in OVA-sensitized mice. Furthermore, the results of MCH-induced airway response tests indicated that R555E treatment markedly suppressed OVA-elicited airway hyperreactivity. These results indicate that Akt activation plays a role in the inflammatory response and that NGF and SH2-B β upregulation of Akt is involved in the neuroimmunological pathogenesis of allergic challenge in mice. These findings may pave the way for the development of new therapeutic approaches to allergic airway diseases.

Nerve growth factor plays an important role in bronchial hyperresponsiveness and inflammation^{21,22} in asthma. This protein is overexpressed in the serum and BALF of asthmatic patients and more highly expressed in the serum of allergic asthmatic patients than in that of non-allergic asthmatic patients.^{23,24} Increased NGF levels have been found in the airways of an asthma animal model,²⁵ as well as in lymphocytes, macrophages, airway epithelial cells, pulmonary fibroblasts and airway smooth muscle cells.^{26,27} NGF has several proinflammatory properties, facilitating mediator release from inflammatory cells, mast-cell infiltration, eosinophil activation and fibroblast activation.²⁸ Our results demonstrated that intravenous administration of anti-NGF antibody can block the proinflammatory properties of NGF and remedy hyperplasia of airway epithelial cells and the alveolar and interstitial oedema caused by allergens. These findings are consistent with those of Günter Páth *et al.*²⁹ using challenged BALB/c mice. Other reports have also shown that nasally administered

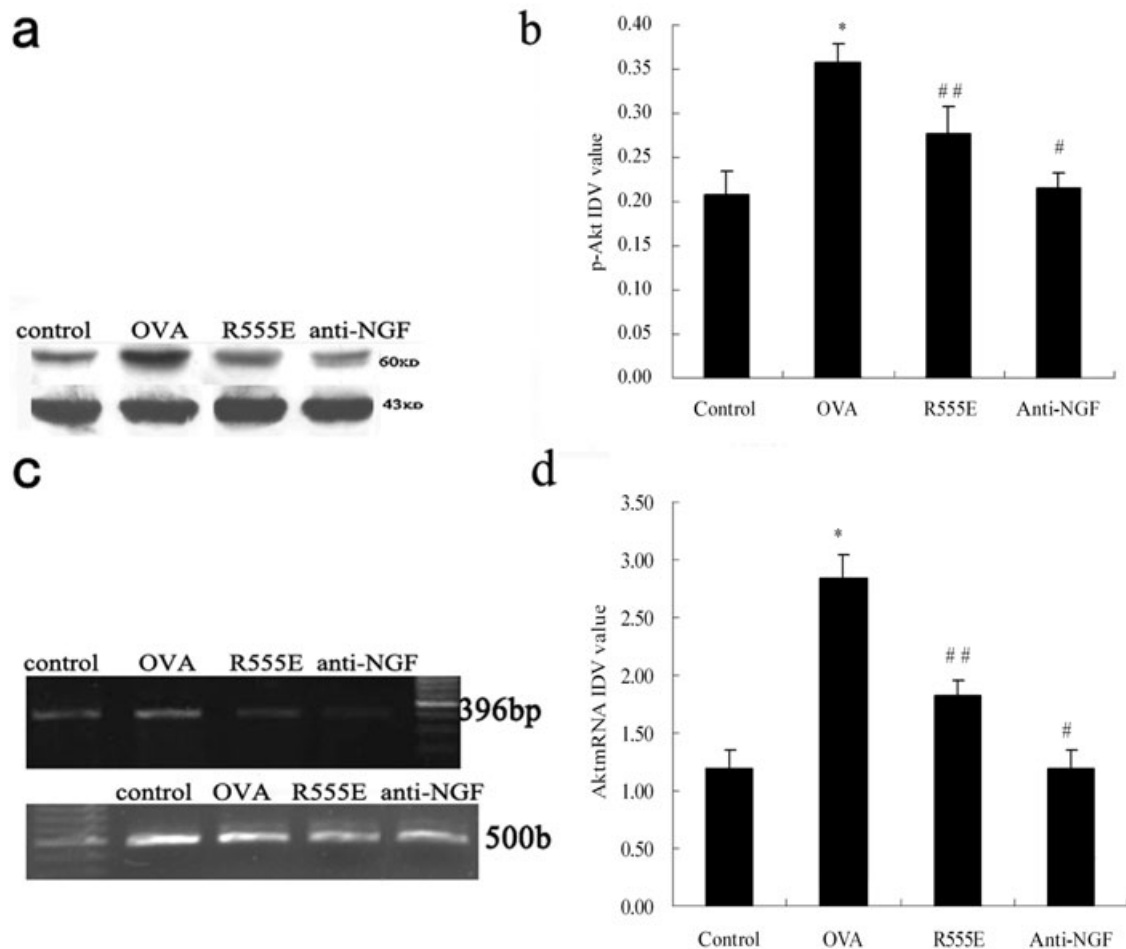


Figure 4 (a) Western blot analysis showing varying expression levels of p-Akt protein in the lung tissue of the (1) control group, (2) ovalbumin (OVA) treated group, (3) R555E group and (4) anti-NGF group. The lower row shows the intensity of a 43-kDa β -actin band in the same immunoblot, which is indicative of protein-loading variance. (b) Changes in the integrated density values ratio of p-Akt protein to β -actin expression. Versus control group * $P < 0.01$, versus OVA group # $P < 0.05$ or ## $P < 0.01$. (c) Representative RT-PCR illustrating differences in the 396-bp Akt band and the 500-bp β -actin band in the lung tissue of the (1) control group, (2) ovalbumin (OVA) treated group, (3) R555E group and (4) anti-NGF group. (d) The integrated density value (IDV) ratio of Akt mRNA to β -actin in the lung tissue. Significant differences were seen in each experimental groups compared with the control group * $P < 0.01$, versus OVA group ## $P < 0.05$ and # $P < 0.01$.

anti-NGF significantly reduces airway hyperresponsiveness (AHR) and inhibits acute bronchoconstriction in allergic asthmatic guinea pigs.^{30,31}

Nerve growth factor stimulates SH2-B β binding to TrkA, resulting in Akt modulation, which is one of the pathways by which NGF regulates the differentiation, survival and multiplication of PC12 cells.^{32,33} SH2-B β is an intracellular positive regulator that activates this pathway^{17,34} and also promotes activation of the phosphatidylinositol 3-kinase (PI3-K) pathway via insulin-receptor substrates 1 and 2.³⁵ We can thus assume that SH2-B β , as a critical signal protein for NGF-mediated upregulation of Akt activation, is important in the NGF-mediated pathogenesis of asthma. Previous results have demonstrated that SH2-B β is overexpressed in the lung tissue of bronchial asthmatic mice³⁶ and that NGF upregulates

SH2-B β expression, indicating that SH2-B β is involved in the pathogenesis of NGF-induced allergic asthma.³⁷ The essential Arg555 within the SH2 domain of SH2-B β was replaced with Glu. This dominant-negative mutant form of SH2-B β , called R555E as previously reported by Rui *et al.*,⁴ blocks NGF-induced neurite outgrowth in PC12 cells, while overexpression of wild-type SH2-B β enhances this outgrowth. The R555E mutant also inhibits SH2-B β support of glial cell line-derived neurotrophic factor GDNF-induced neurite outgrowth in both PC12-GFR α 1-RET cells (that stably overexpress GDNF receptor α 1 and RET) and cultured mesencephalic neurons.¹⁴

In our study, we found that activated Akt was overexpressed in some infiltrative inflammatory cells and lung tissue and that NGF could regulate Akt

activation. This finding indicates that NGF mediation of the Akt signal pathway was involved in the development of allergic airway disease. In addition, R555E and anti-NGF downregulated Akt activation in allergic airway challenge. R555E administration also suppressed allergen-induced AHR and inflammatory markers. This suggests that NGF-induced Akt activation is markedly affected by SH2-B β , which may be an intracellular positive regulator of the signal pathway responsible for the pathogenesis of allergic airway disease. More specifically, SH2-B β may positively regulate NGF-mediated activation of the Akt pathway in allergic airway disease.

We noted that the blocking effects of R555E on asthma airway resistance and Akt expression are significantly weaker than the effects of anti-NGF. One possible explanation is that SH2-B β only partly participates in NGF-induced Akt activity and that NGF initiates more than one pathway of Akt regulation in asthma. PI3-K-Akt pathway may be activated in two ways: via the growth factor receptor or binding protein or via the renin-angiotensin system (Ras) and Pl10 isoform in phosphoinositide 3-kinase signalling. In NGF signalling pathways, PI3-K/Akt can be used as the Ras downstream signalling protein and B-Raf upstream signal protein.³⁸ He *et al.*³⁹ found that mitogen-activated protein kinase kinase-1/2 (MEK1/2) inhibitors can inhibit Akt-pThr308, Akt-pSer473, and Forkhead (Drosophila) homolog (rhabdomyosarcoma) like 1-pThr32, suggesting crosstalk between the PI3-K/Akt and Ras/mitogen-activated protein kinase (MAPK) signalling pathways, although the exact mechanism needs to be confirmed by further studies. In addition, NGF combines two different types of cell surface receptors, tyrosine kinase receptor A (TrkA) and p75 neurotrophin receptor (p75NTR), and p75NTR is independently coupled to the PI3-K/Akt signalling pathway.⁴⁰ The TrkA-p75NTR complex is a high-affinity NGF receptor with two unique features: (i) the ability to integrate the MAPK and Akt pathways to participate in NGF-induced axonal ectogenesis, and (ii) the ability to induce short-term or persistent dual activation of the Akt pathway to promote the long-term survival of cells. Specific molecular mechanisms of NGF engagement of this receptor are still being investigated. These findings raise the possibility that NGF modulates Akt activity in asthma via mechanisms beyond SH2-B β alone.

Using an *in vivo* asthma model, this study demonstrated that increased Akt activation via SH2-B β is one contributor to NGF-induced airway inflammation and AHR. Intravenous administration of anti-NGF or a mutant of SH2-B β effectively blocked the inflammatory cascade, suggesting that blocking NGF and SH2-B β may be beneficial for the treatment of asthma. It is already known that NGF activates various signalling molecules via TrkA, including phospholipase C, PI3-K/Akt, the small G protein Ras and MAPK, all of which have been implicated in asthma. The present study investigated the role of the NGF-PI3-K/Akt pathway in allergic airway disease in mice, but it is likely that other pathways are also involved and require investigation.

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