

ORIGINAL ARTICLE

SH2-B β expression in alveolar macrophages in BAL fluid of asthmatic guinea pigs and its role in NGF-TrkA-mediated asthma

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ABSTRACT

Background and objective: Nerve growth factor (NGF)/tyrosine kinase receptor A (TrkA) signalling may play an important role in the pathogenesis of asthma, and SH2-B β , a TrkA-binding protein, modulates the NGF signalling pathway. In this study, SH2-B β expression in alveolar macrophages (AM) in guinea pig BAL fluid and its role in asthma pathogenesis through the NGF-TrkA signalling pathway were investigated.

Methods: Guinea pigs were randomized into five groups: control, a model of asthma, anti-SH2-B β antibody treatment, anti-NGF antibody treatment and anti-TrkA antibody treatment. The asthmatic model was established in guinea pigs by inhalation of ovalbumin. Specific anti-SH2-B β , anti-NGF and anti-TrkA antibodies were administered and AM were isolated from BAL fluid to assess SH2-B β expression using an immunofluorescence assay. SH2-B β and TrkA protein expression were determined by western blotting, IL-1 β and IL-4 levels in the BAL fluid supernatants were determined by ELISA, and pathological changes in the bronchi and lung tissues were examined by HE staining.

Results: Lymphocyte, eosinophil and total inflammatory cell numbers in BAL fluid were significantly higher in the asthma model group than in the other groups ($P < 0.01$). NGF expression in the asthma model group was significantly higher than that in the PBS control group ($P < 0.01$). SH2-B β was expressed in AM of control animals and expression was significantly higher in the asthma model than in the other groups ($P < 0.01$). TrkA protein expression was significantly higher in the asthma model group than in the PBS group ($P < 0.01$), and treatment with anti-NGF antibody resulted in significant reduction of TrkA expression ($P < 0.01$).

SUMMARY AT A GLANCE

The expression of SH2-B β in alveolar macrophages of asthmatic guinea pigs and the modulation of SH2-B β expression by nerve growth factor and TrkA were investigated. SH2-B β may participate in the pathogenesis of asthma through the NGF-TrkA pathway, possibly representing a new mechanism in asthma.

Conclusions: SH2-B β is expressed in AM of normal guinea pigs, and SH2-B β may participate in asthma pathogenesis through the NGF-TrkA signalling pathway.

Key words: alveolar macrophage, asthma, nerve growth factor, SH2-B β , tyrosine kinase receptor A.

INTRODUCTION

Chronic airway inflammation and airway hyper-responsiveness are important pathophysiological features of asthma. Rather than being independent pathological processes, airway inflammation and airway hyper-responsiveness are interconnected by bidirectional signalling between immune cells and neural cells, and therefore the identification of bidirectional signalling molecules that mediate the cross-talk between the immune and neural cells has become a focus of asthma research. Recent studies have demonstrated that nerve growth factor (NGF) acts as an important mediator in neuro-immune mechanisms of asthma by regulating neuronal plasticity and connecting neuro-immune mechanisms in immune cells and neurons, thereby mediating airway hyper-responsiveness; it induces airway inflammation by causing neurogenic inflammation and amplifying the effects of immune cells.¹ The diverse actions of NGF are mediated by its binding to a specific high-affinity receptor, the tyrosine kinase receptor A (TrkA).^{2,3}

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As a member of the conservative adapter protein family that includes Lnk and APS, SH2-B β is a TrkA-binding protein,^{4,5} the actions of which are closely related to NGF. After activation by NGF, TrkA binds to SH2-B β to cause tyrosine phosphorylation of SH2-B β , which acts on Akt/PKB to stimulate expression of the forkhead transcription factors. These transcription factors induce differentiation and proliferation of PC12 cells, but R555E, a product of a defective SH2-B β protein in which a Glu residue is replaced by Arg, can inhibit this cascade.⁶ SH2-B β is also a positive regulator of AKT/Forkhead pathway activation, an event initiated by NGF through its action on TrkA.⁷ These data suggest the importance of SH2-B β as a signalling molecule in the NGF pathway. NGF and TrkA both participate in the pathogenesis of asthma, but whether SH2-B β is also involved in this process and whether it acts through the NGF-TrkA pathway remain open questions.

Alveolar macrophages (AM) are extensively distributed over the bronchial and alveolar surfaces and initiate a variety of immune and inflammatory reactions.⁸ AM appear to have a versatile role in the pathogenesis of asthma, participating in the immediate asthmatic response (IAR), initiating and maintaining the late asthmatic response (LAR) and mediating airway hyper-responsiveness.⁹ The importance of AM is often understated in investigations of the pathogenesis of asthma, and reports concerning the relationship of AM to asthma pathogenesis remain rare. In most circumstances, NGF and TrkA are secreted and are present in neural cells. However, recent studies have described the expression of these proteins in AM,^{10,11} supporting the concept that AM represent one of the elements that link the immune and nervous systems.

In this study, the role of SH2-B β in the pathogenesis of asthma and the possible pathways involved in this process were investigated. The findings indicate that SH2-B β is expressed in AM and may participate in the pathogenesis of asthma through the NGF-TrkA signalling pathway.

METHODS

All animal experiments and animal manipulation procedures were approved by the ethics committee for animal use and care at the Institute of Education of China Medical University. The guinea pigs were kept in 12 : 12 h light/dark cycles with free access to food and water.

Animals and reagents

Specific pathogen-free albino guinea pigs weighing 250–300 g were provided by the Laboratory Animal Center, College of Basic Medicine, Jilin University. Chicken ovalbumin (grade V) was obtained from Sigma (St Louis, MO, USA). Goat polyclonal anti-SH2-B β , anti-NGF and anti-TrkA antibodies and monkey anti-goat IgG-horseradish peroxidase conjugate were purchased from Santa Cruz Biotechnology Inc. (Santa

Cruz, CA, USA). Rhodamine-labelled rabbit anti-goat IgG was provided by Zhongshan Goldenbridge Biotechnology Co. Ltd. (Beijing, China). Goat anti-guinea pig IL-4 and IL-1 β ELISA kits were purchased from Bionewtrans Pharmaceutical Biotechnology Co. Ltd. (Franklin, MA, USA).

Animal groups and a model of asthma

Sixty-four guinea pigs were randomized into five groups, namely the PBS control group ($n = 10$), the asthmatic model group ($n = 12$) and the anti-SH2-B β ($n = 18$), anti-NGF ($n = 12$) and anti-TrkA ($n = 12$) groups. Animals in the control group received only intraperitoneal PBS by injection (1 mL) followed 14 days later by daily 5-min inhalations of PBS aerosol for five consecutive days. In the asthmatic model group, the guinea pigs were injected peritoneally with 1 mL of a mixture containing 10% chicken ovalbumin and an equivalent volume of aluminium hydroxide gel prepared with PBS, and 14 days later inhaled aerosols of 0.5% ovalbumin in PBS for 3–5 min (till the onset of asthma) daily for five consecutive days. The guinea pigs in the anti-SH2-B β , anti-NGF and anti-TrkA groups were also subjected to ovalbumin sensitization and asthma induction in the same manner. The animals in the anti-SH2-B β group received a daily intravenous injection of anti-SH2-B β antibody (diluted 1 : 25 with PBS) via the cephalic vein in the fore limb or the small saphenous vein in the hind limb, 3 h prior to induction of asthma; those in the anti-NGF group were injected with 4 mL/kg¹² of anti-NGF antibody (diluted 1 : 1000 with PBS, final concentration 0.1 mg/L), 3 h before induction of asthma, and those in the anti-TrkA group received 0.2 mmol/L² of anti-TrkA antibody (200 mL prepared with PBS) via the nasal cavity, 3 h before induction of asthma. All antibodies were administered daily for five consecutive days. All animals were killed within 24 h after the last ovalbumin exposure.

BAL fluid collection and AM purification

Within 24 h after the last ovalbumin exposure, the guinea pigs were anaesthetized with 3% pentobarbital and killed by cardiac puncture. After flushing the pulmonary vascular bed with sterile saline containing 1% heparin, the right lung was ligated and the left lung was lavaged six times with 5 mL of sterile saline, and the BAL fluid (recovery rate >80%) was stored and processed as previously described.

Pathological examination of bronchial and lung tissue

The right lungs of the guinea pigs were lavaged and perfused with 4% paraformaldehyde to allow the pleura to extend and flatten before fixation of the tissue in 4% paraformaldehyde. The tissues were routinely embedded in paraffin and sectioned (5 μ m) for

Haematoxylin–eosin (HE) staining for assessment of the pathological changes in lung and bronchial tissues by microscopy.

ELISA for measurement of BAL fluid IL-1 β and IL-4

ELISA was used to measure the concentrations of IL-1 β and IL-4 in BAL fluid from the different groups of guinea pigs. All ELISA procedures were performed according to the manufacturer's instructions (Bio-newtrans Pharmaceutical Biotechnology Co. Ltd). The sensitivity of both assays was 1 pg/mL.

Immunocytochemical assessment of NGF in AM of the control and asthma model groups

Expression of NGF protein was assessed by the avidin-biotin horseradish peroxidase technique. AM were placed on coverslips and fixed with 4% paraformaldehyde at 4°C for 30 min, then treated serially with 0.2% Triton X-100, 3% H₂O₂, and serum at 37°C for 30 min. Anti-NGF antibody (1 : 200, 0.01 mol/L PBS, pH 7.4) was added, with overnight incubation at 4°C. Antibody was detected by reaction with diaminobenzidine and the expression of NGF in AM was observed by light microscopy. The average optical density was analysed using the MetaMorph DP10/BX41 (UIC/Olympus, US/JP) image analysis system.

Immunofluorescence detection of SH2-B β in AM

Alveolar macrophages from the PBS control and asthmatic model groups were immobilized on coverslips and washed thrice with PBS for 5 min, then incubated with 0.2% Triton X-100 at 37°C for 30 min. After washing thrice with PBS for 5 min, slides were blocked with serum at 37°C for 30 min and treated with anti-SH2-B β primary antibody (1 : 100, 0.01 mol/L PBS, pH 7.4) and incubated at 4°C overnight. The slides were washed (3 $\times$ 5 min) before addition of the fluorescent secondary antibody (tetramethylrhodamine isothiocyanate (TRITC)-labelled rabbit anti-goat IgG) and incubation at 37°C for 30 min in the dark, followed by washing (3 $\times$ 5 min) without exposure to light and sealing of the slides with 50% glycerol. For the negative control, identical procedures were followed except that the primary antibody was replaced with PBS. The slides were then observed and photographed under a fluorescence microscope.

Western blotting

Alveolar macrophages were lysed on ice in cell lysis buffer containing 50 mmol/L Tris-HCl, 150 mmol/L NaCl, 1 mmol/L phenylmethyl sulphonyl fluoride and 1% Triton X-100 (pH 8.0). The cell lysate was

centrifuged and the supernatant collected for western blotting and assayed as reported.

Statistical analysis

Statistical analyses were performed using SPSS 11.5 software. All data are expressed as mean $\pm$ SD. Differences between groups were analysed as appropriate using *t*-tests and one-way analysis of variance followed by Fisher's LSD test. A *P*-value of < 0.05 was considered statistically significant.

RESULTS

Pathological changes in the bronchi and lung tissues

The guinea pigs in the PBS group had normal bronchial and alveolar structures, the mucosal lining of the airways exhibited no obvious swelling, and the bronchial walls remained smooth. The airway and lung tissues showed no inflammatory cell infiltration, and no lymphocytes or eosinophils were present (Fig. 1a). In the guinea pigs in the asthma model group, numerous inflammatory cells, including lymphocytes and eosinophils, had infiltrated the lung tissues and the bronchiolar lumen and walls, and the airway mucosa showed obvious oedema with bronchiolar wall thickening causing luminal stenosis. Mucus plugs were seen in some of the bronchioles, suggesting successful establishment of the asthmatic model (Fig. 1b). In the asthmatic guinea pigs treated with anti-SH2-B β (Fig. 1c), anti-NGF (Fig. 1d) and anti-TrkA antibodies (Fig. 1e), the bronchi and the lung tissues also exhibited pathological changes with narrowed bronchioles, but these changes were obviously milder than those in the asthma model group.

Total cell numbers and differential cell percentages in BAL fluid

The total cell numbers and lymphocyte and eosinophil counts in BAL fluid were significantly greater in the untreated asthmatic guinea pigs than in those treated with anti-NGF, anti-TrkA or anti-SH2-B β antibodies (*P* < 0.01). However, there were no significant differences among the three groups treated with the different antibodies (*P* > 0.05) (Table 1).

IL-1 β and IL-4 concentrations in BAL fluid

IL-1 β and IL-4 levels in BAL fluid were significantly higher in the asthmatic group than in the PBS and anti-SH2-B β groups (*P* < 0.01), providing direct evidence that SH2-B β may affect the expression of inflammatory cytokines, and suggesting the possible involvement of SH2-B β in inflammatory reactions in asthma (Table 2). The levels of IL-1 β and IL-4 in the

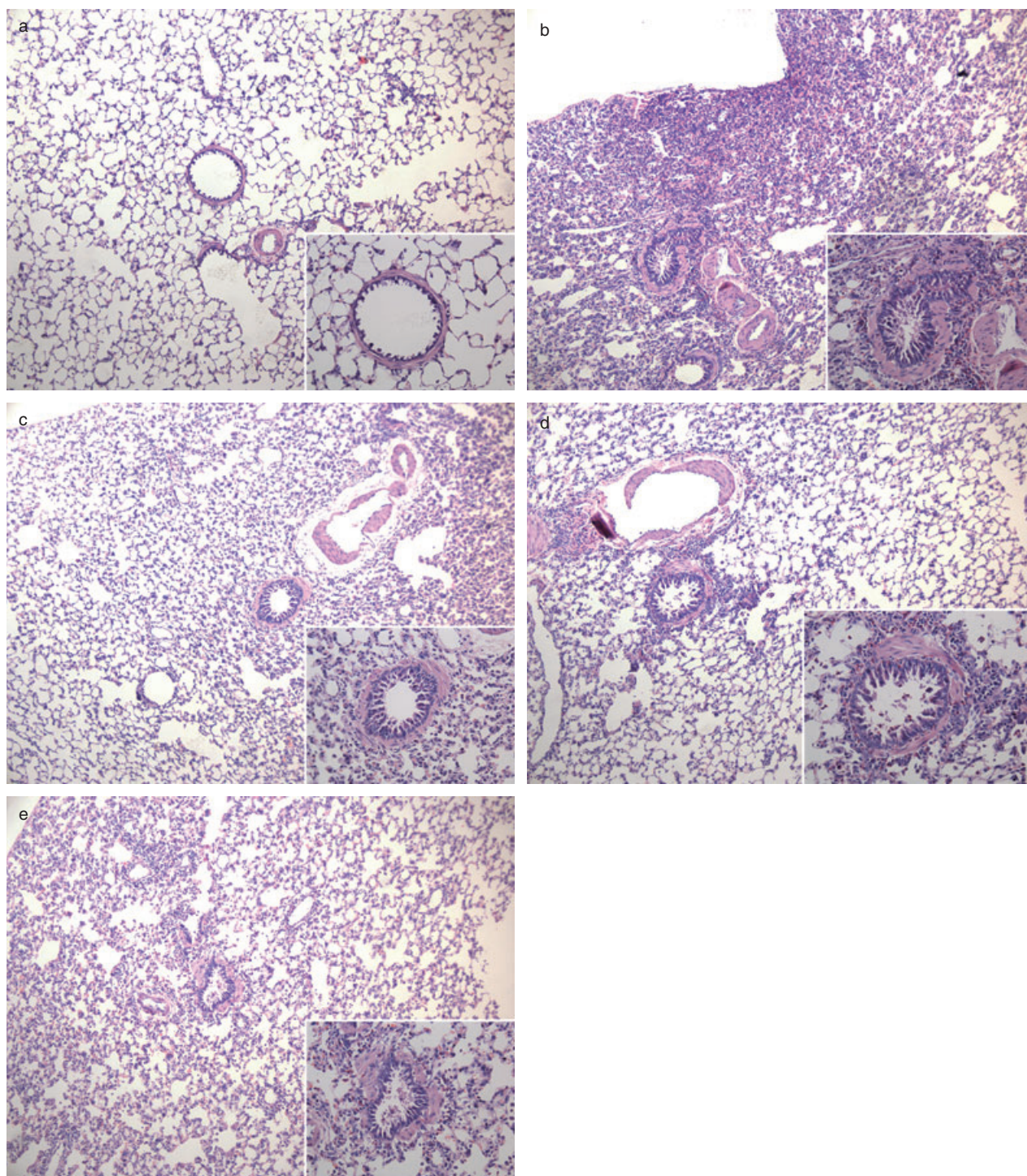


Figure 1 Pathological changes in the bronchi and lung tissues of guinea pigs in the different groups. (a) Normal control group; (b) asthmatic model group; (c) anti-SH2-B β group; (d) anti-NGF group; (e) anti-tyrosine kinase receptor A group. Obvious bronchiolar stenosis can be seen in the asthmatic model group, and the bronchiolar and lung tissue shows obvious inflammatory cell infiltration. (c–e) The alleviated bronchiolar and lung tissue inflammation with significantly relieved bronchiolar narrowing after intervention with the antibodies (HE staining, $\times 100$, $\times 400$, original magnification). NGF, nerve growth factor.

Table 1 Total cell number and the percentages of alveolar macrophages, lymphocytes and eosinophils in BAL fluid of the different groups

Group	Total cell number ($\times 10^7$)	Alveolar macrophages (%)	Lymphocytes (%)	Eosinophils (%)
Control	30.32 $\pm$ 3.47	89.92 $\pm$ 6.73	3.16 $\pm$ 0.29	1.80 $\pm$ 0.21
Asthma	47.33 $\pm$ 4.09*	67.31 $\pm$ 4.20*	9.76 $\pm$ 1.25*	13.74 $\pm$ 0.79*
Anti-NGF	31.97 $\pm$ 2.63***	78.75 $\pm$ 6.28**	4.75 $\pm$ 0.77***	6.41 $\pm$ 0.61***
Anti-TrkA	35.34 $\pm$ 2.31**	81.77 $\pm$ 6.90***	5.36 $\pm$ 0.49***	7.57 $\pm$ 1.03**
Anti-SH2-B β	36.92 $\pm$ 3.57**	82.39 $\pm$ 9.01***	5.73 $\pm$ 1.07**	8.03 $\pm$ 0.75**

* $P < 0.01$ versus control group; ** $P < 0.05$, *** $P < 0.01$ versus asthma group; $n = 7$ or 8.
Values are mean $\pm$ SD.

Table 2 IL-1 β and IL-4 concentrations in BAL fluid supernatants of the different groups

Group	IL-4 (pg/mL)	IL-1 β (pg/mL)
Control	38.44 $\pm$ 3.12	8.55 $\pm$ 0.62
Asthma	81.55 $\pm$ 7.62*	25.70 $\pm$ 3.36*
Anti-NGF	56.83 $\pm$ 4.12***	9.14 $\pm$ 1.05***
Anti-TrkA	59.37 $\pm$ 6.96**	11.92 $\pm$ 1.68***
Anti-SH2-B β	63.28 $\pm$ 7.84**	14.98 $\pm$ 2.93**

* $P < 0.01$ versus control group; ** $P < 0.05$,
*** $P < 0.01$ versus asthma group; $n = 7$ or 8.
Values are mean $\pm$ SD.

anti-NGF and anti-TrkA groups were not significantly different from those in the anti-SH2-B β group ($P > 0.05$) (Table 2).

Immunofluorescence detection of SH2-B β in AM

The red fluorescence emitted by the TRITC-labelled SH2-B β antibody indicated the expression of SH2-B β in the AM. No fluorescence was detected in the negative control slides, in which anti-SH2-B β was replaced with PBS (Fig. 2a), demonstrating the absence of non-specific staining. In the AM from normal guinea pigs treated with anti-SH2-B β antibody, weak fluorescence was observed, mainly on the cell membrane and in the cytoplasm, and occasionally in the cell nuclei (Fig. 2b). The AM from asthmatic guinea pigs exhibited distinct fluorescence in the same cellular regions as normal AM, but with a much greater intensity (Fig. 2c). These findings demonstrated the presence of SH2-B β expression in AM from asthmatic guinea pigs, suggesting the involvement of SH2-B β in the pathogenesis of asthma.

Immunocytochemical detection of NGF in AM

Expression of NGF, mainly in the cytoplasm, was visible as brownish yellow particles (Fig. 2d,e). Optical density analysis showed that expression of NGF in AM

of the control group was significantly lower than in those of the asthmatic model group (Fig. 2f) ($P < 0.05$).

SH2-B β and TrkA expression in AM

Western blotting demonstrated significantly more intense expression of SH2-B β in the AM from asthmatic guinea pigs than in those from control animals ($P < 0.01$). Expression of SH2-B β in guinea pigs treated with anti-NGF or anti-TrkA antibody was significantly lower than that in asthmatic guinea pigs ($P < 0.05$) (Fig. 3).

Similarly, AM from asthmatic guinea pigs also exhibited significantly more intense expression of TrkA than those of PBS-treated guinea pigs ($P < 0.01$). Intervention with anti-NGF antibody resulted in significant reduction of TrkA expression in the AM ($P < 0.01$, Fig. 4).

DISCUSSION

In the present study, NGF expression in AM of the asthmatic model group was significantly higher than in those of the control group, indicating activation of the NGF/TrkA pathway in the asthma model. SH2-B β expression in AM of guinea pigs was detected using an immunofluorescence assay, and was increased in AM of the asthma model. Intervention with an anti-NGF antibody resulted in significant reduction of SH2-B β and TrkA expression in AM of the asthmatic guinea pigs, and treatment with an anti-TrkA antibody also reduced SH2-B β expression in the AM. These findings suggest that SH2-B β expressed in guinea pig AM participates in the pathogenesis of asthma through the NGF-TrkA signalling pathway.

Alveolar macrophages are extensively distributed over the surface of the bronchi and alveoli and release inflammatory mediators such as leucotriene B4 and platelet-activating factor, which are characteristic of type I allergic reactions in the IAR. The release of these factors from AM coincides temporally with bronchiolar contraction, suggesting the involvement of AM not only in the IAR but also in airway hyper-responsiveness. Leucotriene B4 and

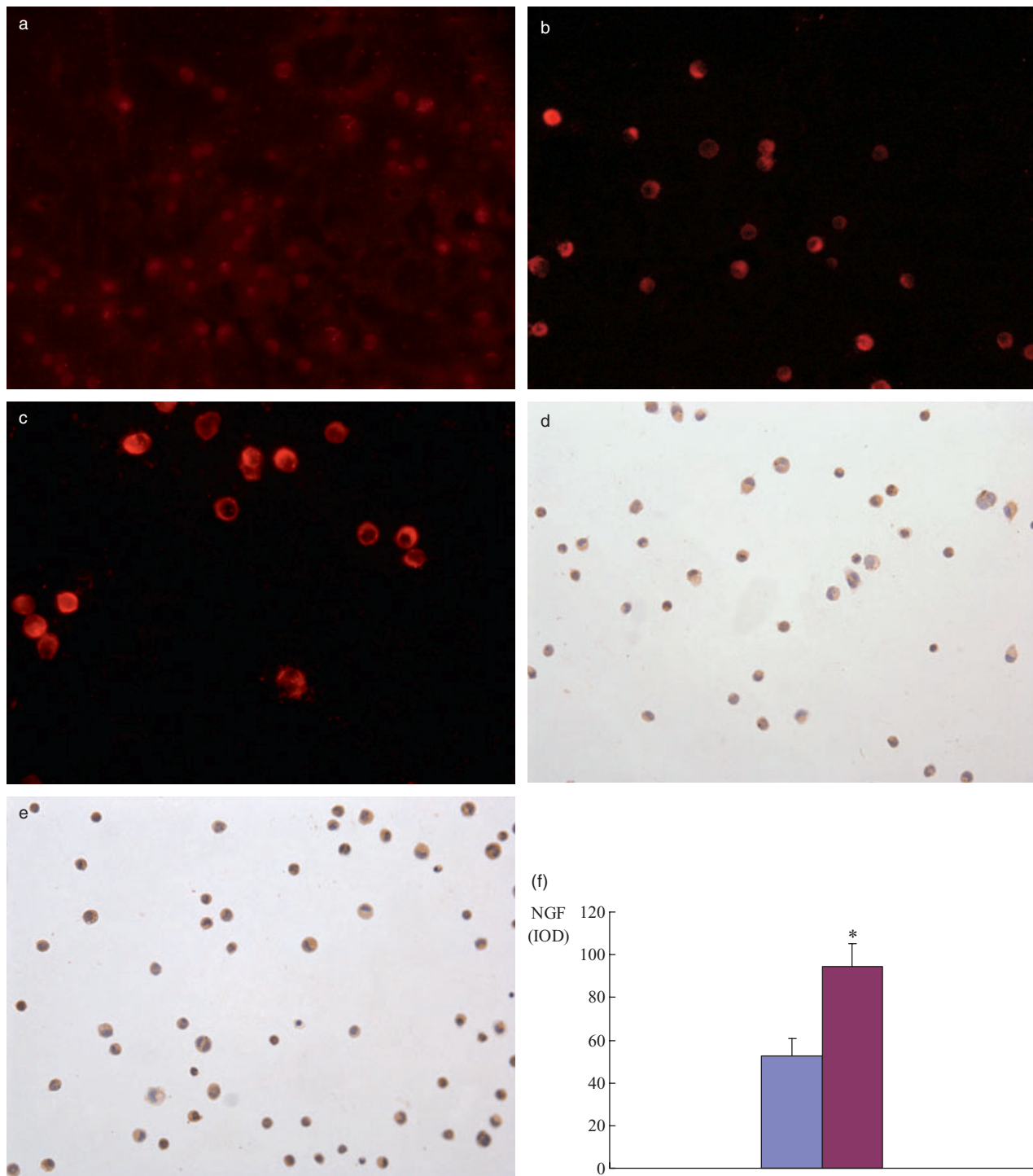


Figure 2 Immunofluorescence detection of SH2-B β expression (a–c) and immunocytochemical detection of nerve growth factor (NGF) expression (d–f) in alveolar macrophages (AM) from normal and asthmatic guinea pigs. (a,d) AM from normal guinea pigs; (b) AM from PBS-treated guinea pigs; (c,e) AM from asthmatic guinea pigs. The red fluorescence emitted by tetramethylrhodamine isothiocyanate-SH2-B β can be seen in the AM, and the fluorescence was obviously more intense in AM from asthmatic than from normal guinea pigs (original magnification, fluorescence microscopy, $\times 400$). NGF-positive AM contain brownish-yellow particles in the cytoplasm. (f) Integrated optical density (IOD) of NGF staining in the AM. The expression of NGF in AM of the normal control group was significantly lower than in those of the asthmatic model group, indicating activation of the NGF/tyrosine kinase receptor A pathway in the asthmatic model group (original magnification, $\times 400$). Data are expressed as mean $\pm$ SEM, $n = 7$ for each group; * $P < 0.05$, (■) asthmatic model group versus (■) control group. NGF, nerve growth factor.

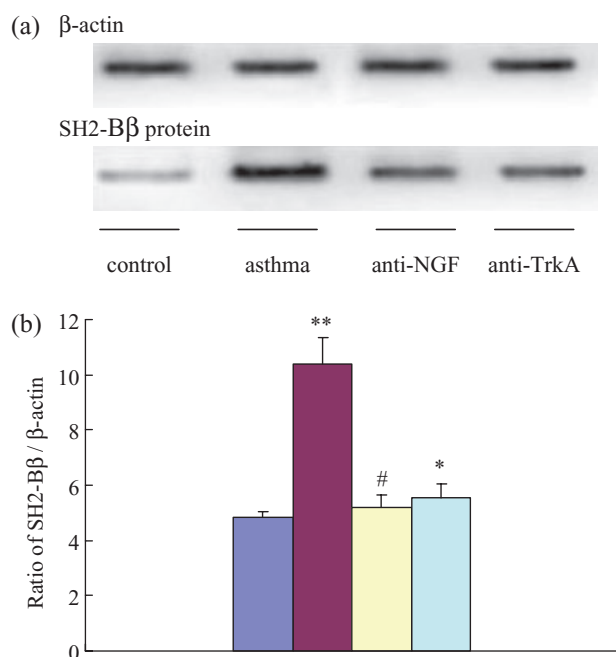


Figure 3 Expression of SH2-B β protein in alveolar macrophages (AM) of guinea pigs as detected by western blotting. (a) Expression of SH2-B β and β -actin in the PBS control, asthmatic model, anti-NGF and anti-TrkA groups; (b) the optical density values for SH2-B β relative to β -actin. Data are expressed as mean $\pm$ SD, $n = 7$ or 8 ; ** $P < 0.01$, (■) asthma versus (■) normal control group; # $P < 0.01$, asthma versus (□) anti-NGF group; * $P < 0.01$, asthma versus (□) anti-TrkA group. NGF, nerve growth factor.

platelet-activating factor are also chemotactic and mobilize the infiltration of inflammatory cells such as eosinophils and lymphocytes into the airways, thereby participating in the initiation of the LAR.⁹ In the LAR, AM release large quantities of inflammatory mediators^{13–15} and pro-inflammatory cytokines such as IL-1 β , IL-8, IL-6 and tumour necrosis factor- α (TNF- α),^{16–18} playing a role in the initiation and progression of airway inflammation. Even in patients with mild asthma, AM show strong pro-inflammatory effects.¹⁹ In the study by Tang *et al.*,²⁰ there was increased eosinophil infiltration of the lung tissues and elevated IL-4 and IL-5 levels in BAL fluid, with the absence of interferon- γ , in mice depleted of AM before ovalbumin challenge to induce asthma. This indicates that AM may function to exacerbate and alleviate inflammatory reactions, behaving as a double-edged sword.⁸ Olgart-Hoglund *et al.*²¹ performed immunohistochemistry to identify the origin of NGF in the airways of asthmatic patients and detected expression of NGF in AM. Considering the previously confirmed presence of TrkA in AM,¹⁰ it can be inferred that AM function as a bridge between the immune and nervous systems. As the most abundant cellular component in BAL fluid, AM are relatively easily isolated and these functionally versatile cells were therefore chosen for this investigation of the pathogenesis of asthma.

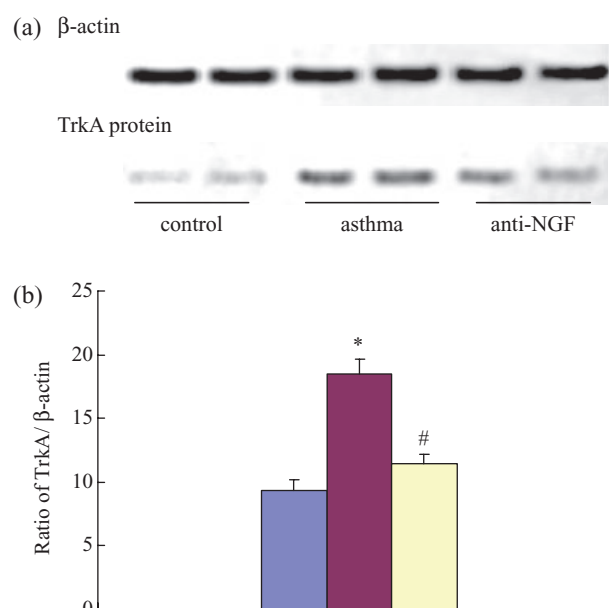


Figure 4 Expression of TrkA protein in alveolar macrophages of guinea pigs as detected by western blotting. (a) Expression of SH2-B β and β -actin in the PBS control, asthmatic model and anti-NGF groups; (b) the optical density values for TrkA relative to β -actin. Data are expressed as mean $\pm$ SD, $n = 7$ or 8 ; * $P < 0.01$, (■) asthma versus (■) normal control group; # $P < 0.01$, asthma versus (□) anti-NGF group. NGF, nerve growth factor.

SH2-B β has been identified as a membrane-bound protein that binds to TrkA in the presence of NGF to trigger the phosphorylation of SH2-B β at multiple serine/threonine residues by mitogen-activated protein kinase kinase/extracellular signal-regulated kinase, which dissociates SH2-B β from the membrane, allowing it to shuttle between the cytoplasm and the nucleus to accomplish signal transduction.²² In this study, an immunofluorescence assay was used to detect SH2-B β expression in AM. SH2-B β was expressed mainly in the cell membrane and cytoplasm, and occasionally in the nuclei. The presence of SH2-B β in the nuclei might be the result of activated membrane ruffling through SH2-B β .

The immunofluorescence assay revealed enhanced SH2-B β expression in AM of asthmatic guinea pigs compared with those from normal guinea pigs. This finding was also confirmed by western blotting, demonstrating the involvement of SH2-B β in the pathogenesis of asthma. In asthmatic guinea pigs treated with an anti-SH2-B β antibody, histopathological examination revealed substantially ameliorated inflammatory cell infiltration in the lung tissues and bronchi, as well as reduced bronchial constriction. SH2-B β blockade was also associated with a significantly reduced total number of inflammatory cells and reduced numbers of lymphocytes and eosinophils in the BAL fluid of asthmatic animals ($P < 0.01$, Table 1). This was presumably the result of depressed

AM activation due to the blocking of SH2-B β , which attenuates the capacity of inflammatory cells such as lymphocytes and eosinophils to migrate to the airways. The results of ELISA confirmed significant reduction of IL-1 β and IL-4 levels in BAL fluid from asthmatic guinea pigs in response to SH2-B β blockade ($P < 0.01$, Table 2). IL-1 β is secreted primarily by activated macrophages,²³ and participates in the initiation of airway hyper-responsiveness.^{24–27} IL-4 is an important cytokine secreted by lymphocytes and eosinophils, and the reduction in the numbers of lymphocytes and eosinophils as a consequence of SH2-B β blockade may have decreased IL-4 levels in BAL fluid from asthmatic guinea pigs. Taken together, these findings suggest that SH2-B β plays a role in the pathogenesis of asthma.

The antibodies against SH2-B β , TrkA and NGF decreased the inflammatory cell (including lymphocyte and eosinophil) numbers and BAL fluid IL-1 β and IL-4 levels in the asthmatic animals, with comparable potencies, suggesting that SH2-B β possesses a similar capacity to that of NGF and TrkA for regulating inflammatory cells and IL-1 β and IL-4 levels in the BAL fluid. This implies that SH2-B β may be in the NGF–TrkA pathway as the sole adapter protein, which, however, remains only a presumption. Due to the limitations of this study, the SH2-B β blocking experiment was not sufficiently specific to yield conclusive evidence for the exact role of SH2-B β in the pathogenesis of asthma. More detailed studies are required to elucidate the molecular mechanism by which SH2-B β participates in asthma pathogenesis, and to gain a better understanding of the effect of SH2-B β on airway inflammation and responsiveness.

Current studies on SH2-B β are often conducted using PC12 cells, in which SH2-B β has been identified as an important molecule in the signalling pathway through which NGF–TrkA binding activates downstream enzymes.^{6,7,22,28} To determine whether SH2-B β plays a similar role in AM of asthmatic guinea pigs, an asthmatic guinea pig model was established, with NGF blockade using a specific antibody. There was substantial suppression of SH2-B β expression in the AM with significantly reduced numbers of inflammatory cells in BAL fluid (Table 1). Diminished numbers of inflammatory cells were also noted in the lung tissues and bronchi of the asthmatic animals following the treatment, with alleviated bronchial wall thickening and bronchiolar stenosis (Fig. 1b,d). TrkA expression was reduced significantly in the AM of asthmatic animals with NGF blockade, suggesting that NGF regulates TrkA and SH2-B β expression in AM, and thereby affects airway inflammation. TrkA blockade produced similar effects, alleviating inflammatory cell infiltration in the bronchiolar and lung tissues, relieving bronchiolar stenosis (Fig. 1b,d), and reducing inflammatory cell (lymphocyte and eosinophil) numbers in BAL fluid (Table 1), indicating that TrkA may also regulate SH2-B β expression and thereby affect the airway inflammatory status. Based on this evidence, we suggest that SH2-B β participates in the NGF–TrkA pathway in AM of asthmatic guinea pigs.

Besides AM, epithelial cells also express NGF and TrkA and may be involved in airway hyper-

responsiveness. However, there are no reports on the expression of SH2-B β in epithelial cells. Some studies have touched on the expression of NGF and TrkA in dendritic cells (DC). Jiang *et al.* showed expression of NGF in dendritic cells.²⁹ la Sala *et al.* showed that mononuclear cells expressed TrkA but not P75NTR; when mononuclear cells differentiated into DC, the expression of TrkA decreased and the cells became insensitive to NGF.³⁰ Therefore, it is speculated that NGF and TrkA in DC may play a limited role in the pathogenesis of asthma. However, we did not find any reports on the expression of SH2-B β in DC. Therefore, whether SH2-B β plays a role in asthma pathogenesis through epithelial cells and DC will be an interesting area for future study.

Currently, inhalation of β_2 -agonists and glucocorticoids is effective for treatment of asthma. Studies have revealed that glucocorticoids exerted the anti-asthmatic effect through decreasing the expression of NGF and TrkA;^{31–35} β_2 -agonists were also found to be capable of downregulating NGF.^{36–38} Further study on the influence of β_2 -agonists and glucocorticoids on expression of SH2-B β may shed new light on the anti-asthmatic mechanisms of β_2 -agonists and glucocorticoids.

In conclusion, the signalling molecule, SH2-B β , is expressed not only in PC12 cells but also in AM of guinea pigs, and may play a role through the NGF–TrkA pathway in the initiation of airway inflammation and hyper-responsiveness in guinea pigs.

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