

Kidins220/ARMS contributes to airway inflammation and hyper-responsiveness in OVA-sensitized mice

Xiuqin Ni^{a,b,1}, Xing Li^{c,1}, Xiubin Fang^{a,*}, Ning Li^a, Wanpeng Cui^a, Baohui Zhang^a, Yuli Liu^a

^a Department of Neurobiology, Institute of Respiratory Diseases, China Medical University, Shenyang 110001, China

^b Department of Anatomy, Daqing Branch of Haerbin Medical University, Daqing 163319, China

^c Department of Nephrology, Daqing People Hospital, Daqing 163310, China

ARTICLE INFO

Article history:

Accepted 17 September 2010

Keywords:

Kidins220/ARMS

Asthma

Ovalbumin

Airway inflammation

Airway hyper-responsiveness

NF- κ B

ABSTRACT

BALB/c mice were sensitized and challenged with ovalbumin. We hypothesized that Kidins220/ARMS influences airway inflammation and hyper-responsiveness during allergic airway challenge, and assessed it by intranasal administration of anti-NGF antibody or anti-ARMS antibody to mice. Airway resistance was measured using a sealed whole-body plethysmograph. Total cell numbers and the percentage of different inflammatory cells in BALF were counted. Expression of IL-1 β , IL-4 and TNF- α were determined by ELISA, and NF- κ B activation determined by EMSA. Kidins220/ARMS expression was observed in ovalbumin-sensitized mice by immunofluorescence or western blotting. IL-1 β , IL-4, and TNF- α were overexpressed and NF- κ B activation increased after allergen challenge compared with controls. After treatment with anti-ARMS or anti-NGF, levels of IL-1 β , IL-4 and TNF- α and NF- κ B activation were reduced in comparison with those of ovalbumin-sensitized mice. These results suggest that NGF-mediated Kidins220/ARMS signaling participates in the pathogenesis of asthma, and contributes to airway inflammation and hyper-responsiveness in ovalbumin-sensitized mice.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Asthma is a complex disease characterized by reversible airway obstruction, airway hyper-responsiveness and chronic inflammation of the airways. Many cell types and cytokines contribute to this inflammation, which enhances bronchial sensitivity to various stimuli (Ying et al., 2006). Nerve growth factor (NGF) has been specifically implicated in the pathogenesis of asthma because it can promote sensory neuron hyper-reactivity and induce airway inflammation (El-Banna et al., 2006; Obermeier et al., 1994). The diverse actions of NGF are mediated by its binding to a specific high-affinity receptor: tyrosine kinase receptor A (TrkA) (De Vries et al., 2006; Freund and Frossard, 2004). In addition, ankyrin-rich membrane-spanning (ARMS) protein/substrate for protein kinase D (Kidins220), a novel transmembrane protein substrate of Trk receptors, is directly involved in the downstream signaling events for neurotrophins (Kong et al., 2001). ARMS has been identified as an important molecule in the signaling pathway through binding with NGF-TrkA for activating downstream enzymes (Edwards et al., 2009). In PC12 cells treated with NGF for brief intervals, tyro-

sine phosphorylation of ARMS occurred simultaneously with the detection of phosphorylated TrkA receptors. Moreover, ARMS plays a critical part in the branching of cortical and hippocampal dendrites, and in the turnover of cortical spines (Wu et al., 2009). ARMS may promote nuclear factor kappa-B (NF- κ B) signaling via activation of mitogen-activated kinase (MAPK) and I κ B kinase (IKK), thereby facilitating phosphorylation of RelA (major NF- κ B subunit) at an IKK-sensitive site. ARMS is a major factor that links neurotrophin signaling to NF- κ B (Sniderhan et al., 2008). Interleukin (IL)-1 β and tumor necrosis factor (TNF)- α have been shown to have a prominent role in the development of airway responsiveness and airway inflammation in bronchial asthma (Gosset et al., 1991; Wills-Karp et al., 1993). IL-4 is a Th2 cytokine that has important roles in allergic inflammation and airway remodeling (Jie et al., 2009; Yoshinaka et al., 2002). Together, these studies suggest that ARMS plays a fundamental part as a signaling molecule in the NGF pathway. NGF and TrkA participate in the pathogenesis of asthma.

Based on the information detailed above, we hypothesized that upregulation of ARMS may contribute to: (i) the pathogenesis of NGF-mediated asthma, and (ii) airway inflammation and hyper-responsiveness in asthma. To test this hypothesis, the role of ARMS/Kidins220 in the pathogenesis of asthma and in airway inflammation and airway hyper-responsiveness was investigated. The findings indicated that Kidins220/ARMS signaling was acti-

* Corresponding author. Tel.: +86 24 23252582; fax: +86 24 23252582.

E-mail address: xbfang@mail.cmu.edu.cn (X. Fang).

¹ These authors contributed equally to this work.

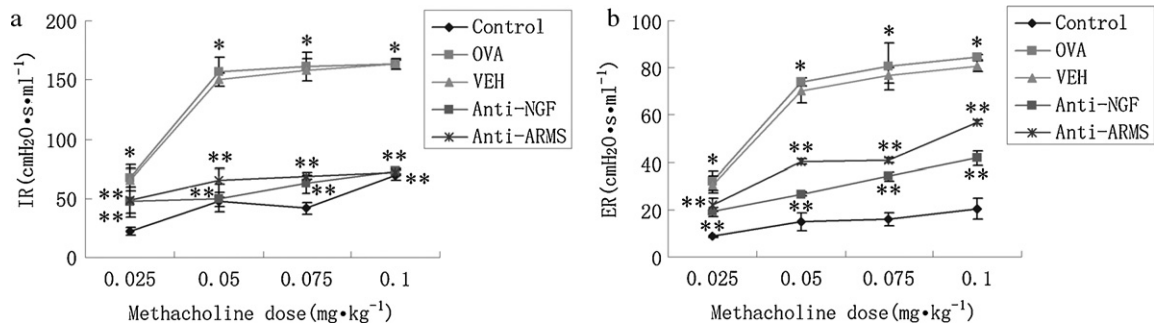


Fig. 1. (a) Airway inspiratory resistance (IR) and (b) airway expiratory resistance (ER) of five groups with regard to MCH dose–response were analyzed using animal lung function apparatus. The dose–response curves showed that the airway response to MCH was increased in the OVA group and VEH group compared with that in the control group. Treatment of OVA-sensitized mice with anti-NGF and anti-ARMS prevented this increase in airway reactivity. * $P < 0.01$ vs control group; ** $P < 0.01$ vs OVA group and VEH group.

vated in ovalbumin (OVA)-sensitized mice and may contribute to airway inflammation and hyper-responsiveness.

2. Materials and methods

2.1. Ethical approval of the study protocol

All animal experiments as well as procedures involving animal manipulation were approved by the Ethics Committee for Animal Use and Care at the Institute of Education of China Medical University (Shenyang, China).

2.2. Animals

Specific pathogen-free female BALB/c mice (age, 6–8 weeks; 18–20 g) were provided by the Laboratory Animal Research Center in Beijing, China. These mice were housed in cages and maintained in a room with a 12-h/12-h light/dark cycle with free access to food and water.

2.3. Sensitization, challenge and experimental protocol

Sixty-four BALB/c mice were randomized into five groups: control, ovalbumin (OVA), vehicle (VEH), anti-NGF, and anti-ARMS. All groups contained 12 mice except the OVA group, which had 16 mice. Sensitization and challenge protocols were carried out according to the methods of Elwood et al. (1991) and Vanacker et al. (2001) with some modifications (as described below). On days 0, 7, 14 and 21, all mice except the control group were actively sensitized with OVA (1 mg, i.p.; Sigma–Aldrich, St Louis, MO, USA) and 100 mg aluminum hydroxide in 0.5 mL phosphate-buffered saline (PBS). Mice in the control group were injected with 0.5 mL PBS

only. Control-group mice were then exposed to aerosolized PBS for 30 min/day from days 22 to 31. Meanwhile, OVA-sensitized mice were exposed to 40 mg/mL aerosolized OVA (4 g OVA in 100 mL sterile PBS in a nebulizer) for 30 min/day from days 22 to 31. Anti-NGF group mice underwent intranasal application of 50 μ L (diluted 1:50 in PBS) polyclonal goat anti-mouse NGF antibody (biological activity: 1:4000 dilution blocks bioactivity of 5 ng/mL NGF) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Intranasal treatment of mice using anti-NGF antibody was undertaken according to the method of Braun et al. (1998), Glaab et al. (2003) and Nagai et al. (2004) with minor modification. Mice in the anti-ARMS group underwent intranasal application of goat polyclonal anti-ARMS antibody (diluted 1:25 in PBS) (Santa Cruz Biotechnology). VEH-group mice underwent intranasal application of PBS. Intranasal treatment was 3 h before allergen aerosol challenge on days 27, 28, 29, 30 and 31. All mice were killed within 24 h after the last exposure to OVA or PBS.

2.4. Determination of airway hypersensitivity

Twenty-four hours after the final challenge, mice were anaesthetized with pentobarbital sodium (80 mg/kg, i.p.). If sensitivity of the tail to pain was disesthesia or anesthesia, the trachea was cannulated and airway resistance measured using an AniRes 2005 Animal Lung Function Analysis machine (Peking Biolab Tech Company, Beijing, China). As described by Liu et al. (2010), Xu et al. (2002) and Zhu et al. (2007), mice were anaesthetized, a tracheotomy was carried out, a cannula connected to a mechanical ventilator, and a signal conditioning unit connected to a sealed whole-body plethysmograph. Mice were then placed in a sealed whole-body plethysmograph and connected to a rodent ventilator at 90 breaths/min. Transducers (ML-AMP II, Peking SYNOL

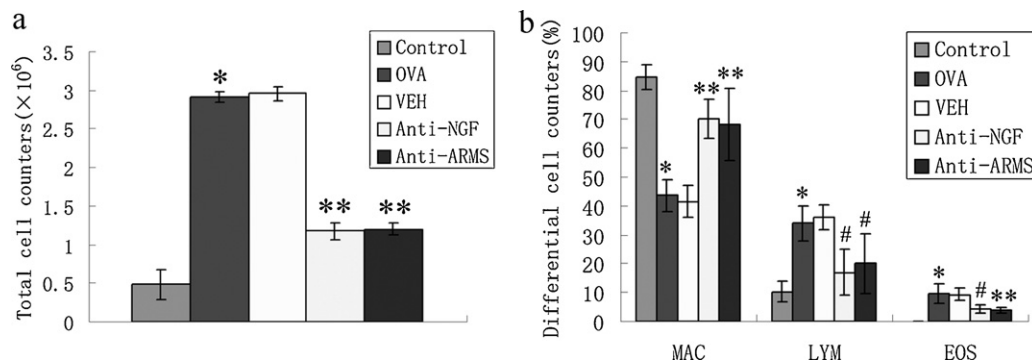


Fig. 2. Counts of total numbers of cells and different cells in BALF of the different groups. Data are mean $\pm$ S.D. ($n = 6$). (a) Total cell counts. * $P < 0.01$ vs control group; ** $P < 0.01$ vs OVA group and VEH group; (b) Differential cell counts. MAC, macrophages; LYM, lymphocytes and EOS, eosinophils. * $P < 0.01$ vs control group; ** $P < 0.01$ or # $P < 0.05$ vs OVA group and VEH group.

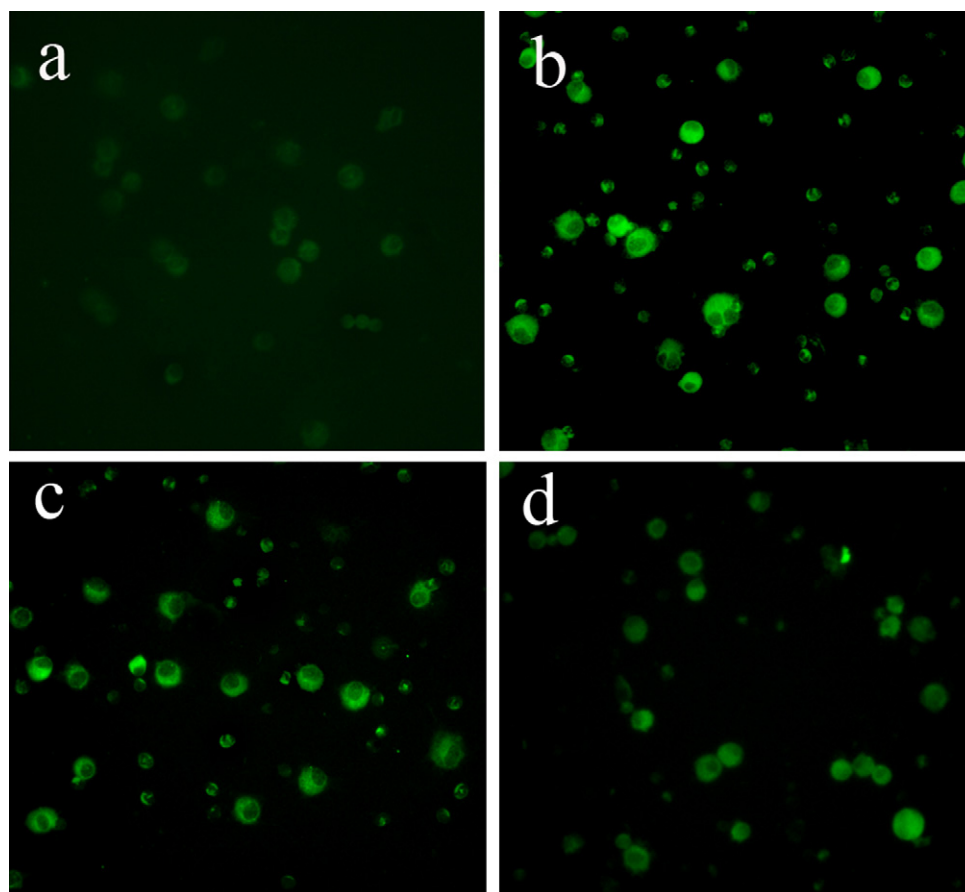


Fig. 3. Localization of ARMS in BALF cells from (a) control, (b) OVA, (c) VEH and (d) anti-NGF group by immunofluorescence. Representative images ($n = 4$ experiments) are shown of ARMS staining (green). The green fluorescence was clearly more intense in BALF cells from the OVA and VEH group mice than that from control mice and anti-NGF treatment mice (original magnification, fluorescence microscopy, $\times 400$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

High-Tech Company, Beijing, China) were connected to the ventilatory circuit. This provided voltage signals for pressure and flow, which were amplified and recorded for subsequent analyses. A microcomputer running AniRes 2005 software was used to continuously calculate the inspiratory resistance (IR) and expiratory resistance (ER) of the respiratory system from the digitized signals of pressure and flow. After stabilization of respiratory parameters, mice were given methacholine (MCH) dissolved in 0.9% sodium chloride; i.v.; Sigma–Aldrich) 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 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 increased above the baseline immediately after MCH administration.

2.5. Bronchoalveolar lavage fluid (BALF)

Mice were killed 24 h after the final OVA challenge, followed by BALF collection using a previously described method (Kim et al., 2007). Three aliquots of 1-mL PBS were injected and withdrawn through the tracheal cannula (recovery rate >75–80%). After centrifugation at $500 \times g$, the supernatant was stored at -80°C for further measurement of concentrations of IL-1 β , IL-4 and TNF- α using an enzyme-linked immunosorbent assay (ELISA). Total cell numbers were counted with a hemacytometer. Cytospin slides of BALF cells were prepared using Cytospin II (Shandon, Runcorn,

UK) and stained with Wright–Giemsa. Differential cell counts were determined by light microscopy (Olympus, Japan) from a count of ≤ 200 cells. All slides were counted twice by different observers blinded to the study protocol.

2.6. ELISA for measurement of the concentration of IL-1 β , IL-4 and TNF- α in BALF

ELISA was used to measure the concentrations of IL-1 β , IL-4 and TNF- α in BALF in the five groups. All ELISA procedures were undertaken according to manufacturer's instructions (Bionewtrans Pharmaceutical Biotechnology Company Limited, Franklin, MA, USA). The sensitivity of these assays to IL-1 β , IL-4, and TNF- α was 1, 1, and 7.8 pg/mL, respectively.

2.7. Immunolocalization of Kidins220/ARMS

Smears of BALF cells from the control, OVA, VEH and anti-NGF group were fixed with cold acetone for 10 min and washed thrice with PBS for 5 min. They were then incubated with 0.2% Triton X-100 at 37°C for 30 min. After being washed thrice with PBS for 5 min, slides were blocked with cow serum at 37°C for 30 min and then incubated with 1:50 goat polyclonal anti-ARMS antibody (Santa Cruz Biotechnology) at 4°C overnight. After being washed thrice with PBS, slides were incubated with 1:200 cow anti-goat IgG conjugated to fluorescein isothiocyanate (Santa Cruz Biotechnology) at 37°C for 30 min in the dark. Slides were then mounted with glycerol and images captured using an Olym-

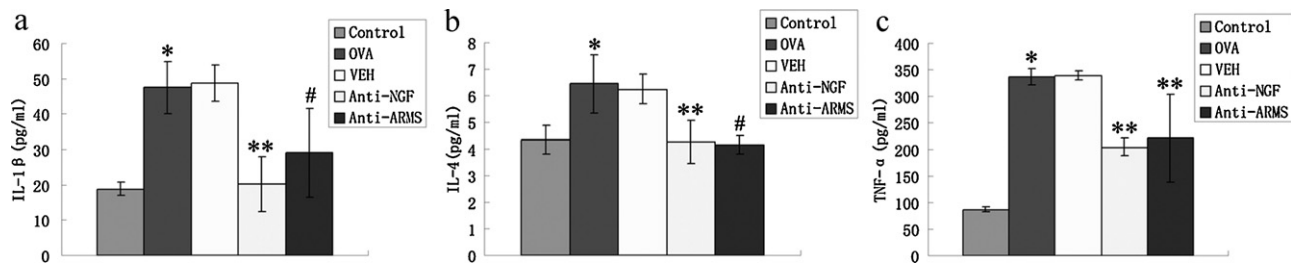


Fig. 4. Effect of anti-ARMS treatment on levels of IL-1β, IL-4 and TNF-α. Levels of (a) IL-1β, (b) IL-4 and (c) TNF-α in the supernatant of BALF were measured by ELISA. Data are mean ± S.D. (*n* = 6). **P* < 0.01 vs control group; ***P* < 0.01 or #*P* < 0.05 vs OVA group and VEH group.

pus BX 60 Upright Fluorescence Microscope (Olympus, Tokyo, Japan).

2.8. Western blot analyses

Protein homogenates of samples of lung tissue were prepared by rapid homogenization in 10 volumes of ice-cold RIPA Lysis Buffer (Beyotime, Shanghai, China). Samples were centrifuged at 17,000 × *g* for 1 h. The protein concentration of the soluble material was determined using an Enhanced Bicinchoninic Acid (BCA) Protein Assay Kit (Beyotime, Shanghai China). Ten micrograms of proteins from each sample were then loaded onto a 7.5% sodium dodecyl sulfate-polyacrylamide gel. Electrophoreted proteins were transferred from the gel to polyvinylidene difluoride membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). After blocking, blots were incubated with 1:1000 goat polyclonal anti-ARMS antibody (Santa Cruz Biotechnology) overnight at 4 °C and further incubated for 1 h with horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biotechnology). The ARMS band was visualized using an Enhanced Chemiluminescence Kit (Santa Cruz Biotechnology). Integrated density values (IDV) were analyzed using a computerized image analysis system (Fluor Chen 2.0) and normalized to those of β-actin.

2.9. Electrophoretic mobility shift assay (EMSA)

Preparation of nuclear extracts and gel mobility shift assays were carried out according to previously described methods (Yin et al., 2008). NF-κB DNA binding activity was measured by EMSA using nuclear extracts from lung tissues. The biotin 5' end-labeled duplex

NF-κB oligonucleotide (5'-AGTTGAGGGGACTTCCAGGC-3') was used as the probe, according to the LightShift Chemiluminescent EMSA Kit (Pierce Biotechnology Incorporated, Rockford, IL, USA). Incubations were done in a buffer containing 10 mM Tris (pH 7.6), 2.5% glycerol, 0.05% Nonidet P-40, and 50 ng/μL of poly (dI-dC). This reaction was then subjected to gel electrophoresis on a native (6%) polyacrylamide gel and transferred to a nylon membrane. The biotin end-labeled DNA was detected using the peroxidase-conjugated streptavidin system. For the cold competition assay, unlabeled probe was added in 500-fold molar excess. The membrane was exposed to X-ray film at room temperature for 3–5 min. NF-κB activity was examined by the optical density (OD) of the band.

2.10. Statistical analyses

Data were analyzed using SPSS 15.0 software (SPSS, Chicago, IL, USA). Data are means ± S.D. Differences between groups were analyzed using the Student's *t*-test and one-way analysis of variance (ANOVA) followed by Fisher's least significant difference test, whereas two-way ANOVA was used to assess differences in airway resistance. *P* < 0.05 was considered significant.

3. Results

3.1. Measurement of airway resistance

All experimental mice exhibited dose-dependent augmentation of IR and ER in response to increasing doses of intravenously administered MCH. There was no statistically significant difference in baseline values of IR and ER among all experimental groups. The airway response to MCH was increased in the OVA group compared with that in the control group (*P* < 0.01). Treatment of OVA-sensitized mice with intranasal anti-NGF and anti-ARMS before OVA challenge prevented this increase in airway reactivity, reflected by a shift in the dose-response curves (Fig. 1a and b) and significantly decreased reactivity compared with the untreated OVA-sensitized mice and VEH-group mice (*P* < 0.01).

3.2. Total cell numbers and differential cell percentages in BALF

The total cell numbers (Fig. 2a) and the percentage of macrophages, lymphocytes and eosinophils (Fig. 2b) in BALF in mice of the OVA group were significantly increased compared with the numbers in mice of the control group (*P* < 0.01). The increased numbers of inflammatory cells (including eosinophils) were significantly reduced by the administration of anti-NGF, anti-ARMS antibodies, as compared with the OVA group and VEH group (*P* < 0.01 and *P* < 0.05). However, there were no significant differences among the two groups when treated with different antibodies (*P* > 0.05).

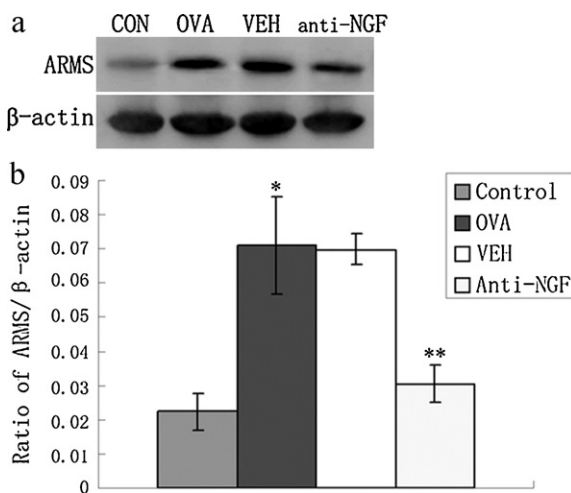


Fig. 5. Expression of ARMS protein in lung tissue by western blotting. (a) Expression of ARMS and β-actin in the control, OVA, VEH and anti-NGF group; (b) optical density values for ARMS relative to β-actin. Data are mean ± S.D. (*n* = 6). **P* < 0.01 vs control group; ***P* < 0.01 vs OVA group and VEH group.

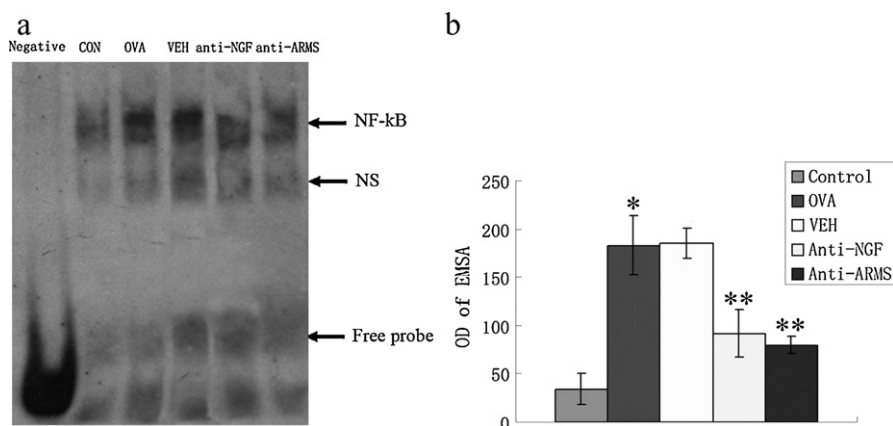


Fig. 6. Identification of activated NF- κ B in the DNA–protein complex by EMSA. Nuclear extracts (7 μ g) from lung in each group were incubated with Binding Reaction. The DNA–protein complexes and unbound probe (free probe) were separated by electrophoretic means on 6.5% polyacrylamide gel. NS indicates a non-specific DNA-binding band. (a) The optical density of EMSA in the control, OVA, VEH, anti-NGF and anti-ARMS group; (b) data are mean $\pm$ S.D. ($n = 6$). * $P < 0.01$ vs control group; ** $P < 0.01$ vs OVA group and VEH group.

3.3. Immunofluorescence detection of ARMS in BALF

The green fluorescence emitted by the FITC-labeled ARMS antibody indicated the expression of ARMS in cells of BALF. Fluorescence was not detected in the negative control slides (in which anti-ARMS was replaced with PBS), demonstrating the absence of non-specific staining. In cells of BALF in the control group, weak fluorescence was observed mainly on the cell membrane and in the cytoplasm, and occasionally in cell nuclei (Fig. 3a). The cells of BALF from the OVA group and VEH group exhibited distinct fluorescence in the same cellular regions as those in the control group, but with a much greater intensity (Fig. 3b and c). The overexpression of ARMS protein at the site stated above was reduced in the anti-NGF group (Fig. 3d). These findings demonstrated ARMS expression in cells of BALF from the OVA group, which may be relevant to NGF.

3.4. Concentrations of IL-1 β , IL-4 and TNF- α in BALF

Levels of IL-1 β , IL-4 and TNF- α in BALF were significantly higher in the OVA group than in the control and anti-ARMS groups ($P < 0.01$). This was direct evidence that ARMS may affect the expression of inflammatory cytokines, and suggested the possible involvement of ARMS in inflammatory reactions. Levels of IL-1 β , IL-4 and TNF- α in the anti-NGF and anti-ARMS group were significantly reduced compared with those in the OVA group and VEH group ($P < 0.01$ and $P < 0.05$) (Fig. 4a–c).

3.5. Expression of ARMS protein

Western blot analyses were carried out to study levels of ARMS protein (Fig. 5a and b) in each group. ARMS protein was expressed at low levels in the control group. However, the levels were markedly increased in the OVA group and were higher significantly than those in the control mice ($P < 0.01$). Expression of ARMS in the anti-NGF group was significantly lower than that in the OVA group and VEH group ($P < 0.01$).

3.6. Activation of NF- κ B

The activation of NF- κ B was measured by EMSA. NF- κ B DNA binding activity was remarkably enhanced in lung tissue from ovalbumin-sensitized mice compared with that in control mice ($P < 0.01$) (Fig. 6a and b). However, mice treated with anti-NGF and anti-ARMS before OVA challenge exhibited less NF- κ B DNA binding activity compared with untreated OVA-sensitized mice

and VEH-group mice ($P < 0.01$). However, there were no significant differences among the two groups treated with anti-NGF and anti-ARMS antibody ($P > 0.05$).

4. Discussion

Accumulating evidences show that NGF plays an important part in bronchial hyper-responsiveness and inflammation (Frossard et al., 2005; Nockher and Renz, 2006) in asthma. NGF levels are increased in the serum and BALF from asthmatic patients compared with those from control subjects (Bonini et al., 1996; Virchow et al., 1998). Kidins220/ARMS was recently shown to be phosphorylated after neurotrophin treatment in PC12 cells and cortical neurons, leading to a prolonged response of MAP kinase (Arevalo et al., 2004). ARMS protein serves as an important downstream platform for the association of ARMS with Trk receptors, through which CrkL–C3G–Rap1 proteins are assembled. Prolonged activation of MAP kinase is facilitated by the formation of this complex (Arevalo et al., 2004). However, whether ARMS is involved in the pathogenesis of asthma and, if so, its mechanism of action, have not been investigated.

In the present study, the expression of ARMS protein in lung tissue as measured by western blot analyses in the OVA group was increased compared with that in the control group. ARMS expression in BALF cells detected using an immunofluorescent assay was increased in the OVA group. Intervention with an anti-NGF antibody resulted in significant reduction of ARMS expression in BALF cells in the OVA group. ARMS blockade was also associated with reduced total cell numbers and numbers of inflammatory cells, including lymphocytes and eosinophils in the BALF of OVA-sensitized mice. This was presumably the result of depressed expression of NGF due to the blocking of ARMS (which attenuates the capacity of inflammatory cells such as lymphocytes and eosinophils to migrate to the airways). These findings suggest that ARMS expressed in BALB/c mice participates in the pathogenesis of asthma through a NGF-mediated signaling pathway.

Some *in vitro* and *in vivo* studies (De Vries et al., 2006; Kerzel et al., 2003; Nassenstein et al., 2006) have suggested that NGF, through its TrkA and p75NTR receptors, might play a part in airway inflammation and in bronchial responsiveness, which are important features observed in allergic diseases of the airway and asthma. Increased NGF levels were found in the airways in animal models of asthma (Virchow et al., 1998) as well as in lymphocytes, macrophages, airway epithelial cells, pulmonary fibroblasts and airway smooth muscle cells (Dinh et al., 2004; Freund and

Frossard, 2004). NGF has pro-inflammatory properties, including mediator release from inflammatory cells, infiltration of mast cells, activation of eosinophils, and induction of fibroblast activation (Olgart Höglund et al., 2002). Kidins220/ARMS represents the only membrane-associated protein known to interact with Trk and p75 receptors (Chang et al., 2004). ARMS facilitates the interaction of Trk receptors with CrkL and Rap-1 proteins, thereby promoting neurotrophin-dependent activation of MAPK (Arevalo et al., 2004). ARMS might promote NF- κ B through a MAPK mechanism (Sniderhan et al., 2008). We can therefore assume that the NGF-TrkA-ARMS signaling pathway may play a part in the inflammation and hyper-responsiveness seen in asthma by activating NF- κ B.

Specific cytokines have been associated with human asthma through studies in blood, exhaled breath condensates, BALF cells or sputum cells (Finkelman et al., 2010). The administration of IL-1 β and TNF- α can increase the airway responsiveness to methacholine and induce the accumulation of neutrophils and alveolar macrophages (Broide et al., 1992; Tillie-Leblond et al., 1999). These cytokines have been detected in increased amounts in BALF (Tillie-Leblond et al., 1999) and in the culture supernatants of alveolar macrophages from asthmatic patients (Azevedo et al., 1997; Gosset et al., 1991). Moreover, the level of the pro-inflammatory cytokine IL-4 is increased in the lung tissues of asthmatic humans (Zimmermann et al., 2003) and animals (Secor et al., 2008; Yin et al., 2008). The cytokines IL-1 β , IL-4 and TNF- α are key players in airway inflammation and airway hyper-responsiveness in allergic asthma. Activation of the transcription factor NF- κ B is linked to the expression of various proteins, including cytokines (Penido et al., 2006; Yin et al., 2008). Th1-associated IL-1 β and TNF- α as well as Th2-associated IL-4 are believed to be the key cytokines involved in the induction of airway hyper-responsiveness, infiltration of eosinophils into the lungs, and mucus secretion (Nakae et al., 2007). In the present study, expression of IL-1 β , IL-4 and TNF- α in BALF in OVA-sensitized mice was higher than that in control mice. We observed activation of NF- κ B in the lung tissue of OVA-sensitized mice, clearly indicating the nuclear translocation of NF- κ B. These results were similar to those of studies in asthmatic patients (Tillie-Leblond et al., 1999; Wright and Christman, 2003; Zimmermann et al., 2003). However, treatment with anti-ARMS antibody resulted in downregulation of expression of NF- κ B, IL-1 β , IL-4, and TNF- α in OVA-sensitized mice. These results suggested that a NGF-mediated ARMS-dependent signaling pathway activated by airway inflammation and airway hyper-responsiveness in allergic asthma may be involved in the mechanism of pathogenesis of asthma through upregulation of NF- κ B, IL-1 β , IL-4 and TNF- α . The present study suggests that an ARMS-blocked method may be applicable for the treatment of airway inflammation and airway hyper-responsiveness in allergic asthma. Furthermore, MCH-induced airway response tests indicated that treatment with anti-ARMS and anti-NGF markedly suppressed OVA-elicited airway hyper-reactivity. These results indicate that ARMS has a role in the inflammatory response and that ARMS upregulation 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 diseases of the airways.

In conclusion, this study demonstrated that NGF-mediated Kidins220/ARMS signaling contributes to airway inflammation and hyper-responsiveness in OVA-sensitized mice. Intranasal administration of anti-NGF or anti-ARMS effectively blocked the inflammatory cascade, suggesting that blocking NGF and ARMS may be beneficial for asthma treatment. It is already known that NGF activates various signaling molecules via TrkA, including phospholipase C, PI3-K/Akt, the small G-protein Ras and MAPK, all of which have been implicated in asthma. We investigated the role of

the NGF-ARMS pathway in allergic airway disease in mice, but it is likely that other pathways are also involved and require investigation.

Acknowledgement

This work was supported by the Natural Science Foundation of China (contract number 30570811 and 81070011).

References

- Arevalo, J.C., Yano, H., Teng, K.K., Chao, M.V., 2004. A unique pathway for sustained neurotrophin signaling through an ankyrin-rich membrane-spanning protein. *EMBO J.* 23, 2358–2368.
- Azevedo, I., de Blic, J., Dumarey, C.H., Scheinmann, P., Vargaftig, B.B., Bachelet, M., 1997. Increased spontaneous release of tumour necrosis factor- α by alveolar macrophages from wheezy infants. *Eur. Respir. J.* 10, 1767–1773.
- Bonini, S., Lambiasi, A., Bonini, S., Angelucci, F., Magrini, L., Manni, L., Aloe, L., 1996. Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc. Natl. Acad. Sci. U.S.A.* 93, 10955–10960.
- Braun, A., Appel, E., Baruch, R., Herz, U., Botchkarev, V., Paus, R., Brodie, C., Renz, H., 1998. Role of nerve growth factor in a mouse model of allergic airway inflammation and asthma. *Eur. J. Immunol.* 28, 3240–3251.
- Broide, D.H., Lotz, M., Cuomo, A.J., Coburn, D.A., Federman, E.C., Wasserman, S.I., 1992. Cytokines in symptomatic asthma airways. *J. Allergy Clin. Immunol.* 89, 958–967.
- Chang, M.S., Arevalo, J.C., Chao, M.V., 2004. Ternary complex with Trk, p75, and an ankyrin-rich membrane spanning protein. *J. Neurosci. Res.* 78, 186–192.
- De Vries, A., Engels, F., Henricks, P.A., Leusink-Muis, T., McGregor, G.P., Braun, A., Groneberg, D.A., Dessing, M.C., Nijkamp, F.P., Fischer, A., 2006. 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. *Clin. Exp. Allergy* 36, 1192–1200.
- Dinh, Q.T., Groneberg, D.A., Peiser, C., 2004. Nerve growth factor-induced substance P in capsaicin-insensitive vagal neurons innervating the lower mouse airway. *Clin. Exp. Allergy* 34, 1474–1479.
- Edwards, M.R., Bartlett, N.W., Clarke, D., Birrell, M., Belvisi, M., Johnston, S.L., 2009. Targeting the NF- κ B pathway in asthma and chronic obstructive pulmonary disease. *Pharmacol. Ther.* 121, 1–13.
- El-Banna, S.M., Mahdi, W.K., Ali, B.A., Raouf, M.M., 2006. Role of nerve growth factor in allergic and inflammatory lung diseases. *J. Microbiol. Immunol. Infect.* 39, 444–451.
- Elwood, W., Lotvall, J.O., Barnes, P.J., Chung, K.F., 1991. Characterization of allergen-induced bronchial hyperresponsiveness and airway inflammation in actively sensitized brown-Norway rats. *J. Allergy Clin. Immunol.* 88, 951–960.
- Finkelman, F.D., Hogan, S.P., Hershey, G.K., Rothenberg, M.E., Wills-Karp, M., 2010. Importance of cytokines in murine allergic airway disease and human asthma. *J. Immunol.* 184, 1663–1674.
- Freund, V., Frossard, N., 2004. Nerve growth factor (NGF) in inflammation and asthma. *Rev. Mal. Respir.* 21, 328–342.
- Frossard, N., Naline, E., Olgart Höglund, C., Georges, O., Advenier, C., 2005. Nerve growth factor is released by IL-1 β and induces hyperresponsiveness of the human isolated bronchus. *Eur. Respir. J.* 26, 15–20.
- Glaab, T., Hoymann, H.G., Hecht, M., Korolewitz, R., Tschernig, T., Hohlfield, J.M., Krug, N., Braun, A., 2003. Effect of anti-nerve growth factor on early and late airway responses in allergic rats. *Allergy* 58, 900–904.
- Gosset, P., Tsicopoulos, A., Wallaert, B., Vannimenus, C., Joseph, M., Tonnel, A.B., Capron, A., 1991. Increased secretion of tumor necrosis factor alpha and interleukin-6 by alveolar macrophages consecutive to the development of the late asthmatic reaction. *J. Allergy Clin. Immunol.* 88, 561–571.
- Jie, Z., Jin, M., Cai, Y., Bai, C., Shen, Y., Yuan, Z., Hu, Y., Holgate, S., 2009. The effects of Th2 cytokines on the expression of ADAM33 in allergen-induced chronic airway inflammation. *Respir. Physiol. Neurobiol.* 168, 289–294.
- Kerzel, S., Path, G., Nockher, W.A., Quarcoo, D., Raap, U., Groneberg, D.A., Dinh, Q.T., Fischer, A., Braun, A., Renz, H., 2003. Pan-neurotrophin receptor p75 contributes to neuronal hyperreactivity and airway inflammation in a murine model of experimental asthma. *Am. J. Respir. Cell Mol. Biol.* 28, 170–178.
- Kim, D.Y., Ryu, S.Y., Lim, J.E., Lee, Y.S., Ro, J.Y., 2007. Anti-inflammatory mechanism of simvastatin in mouse allergic asthma model. *Eur. J. Pharmacol.* 557, 76–86.
- Kong, H., Boulter, J., Weber, J.L., Lai, C., Chao, M.V., 2001. An evolutionarily conserved transmembrane protein that is a novel downstream target of neurotrophin and ephrin receptors. *J. Neurosci.* 21, 176–185.
- Liu, Y., Zhang, B., Zhang, S., Qi, J., Zhang, Z., Liu, L., Fang, X., 2010. Nerve growth factor mediated SH₂-B β /Akt signal pathway activated in allergic airway challenge in mice. *Respirology* 15, 80–87.
- Nakae, S., Lunderius, C., Ho, L.H., Schafer, B., Tsai, M., Galli, S.J., 2007. TNF can contribute to multiple features of ovalbumin-induced allergic inflammation of the airways in mice. *J. Allergy Clin. Immunol.* 119, 680–686.
- Nagai, T., Arai, Y., Emori, M., Nunome, S.Y., Yabe, T., Takeda, T., Yamada, H., 2004. Anti-allergic activity of a Kampo (Japanese herbal) medicine “Sho-seiryuto” (Xiao-Qing-Long-Tang) on airway inflammation in a mouse model. *Int. Immunopharmacol.* 4, 1353–1365.

- Nassenstein, C., Dawbarn, D., Pollock, K., Allen, S.J., Erpenbeck, V.J., Spies, E., Krug, N., Braun, A., 2006. Pulmonary distribution, regulation, and functional role of Trk receptors in a murine model of asthma. *J. Allergy Clin. Immunol.* 118, 597–605.
- Nockher, W.A., Renz, H., 2006. Neurotrophins in allergic diseases: from neuronal growth factors to intercellular signaling molecules. *J. Allergy Clin. Immunol.* 117, 583–589.
- Obermeier, A., Bradshaw, R.A., Seedorf, K., Choidas, A., Schlessinger, J., Ullrich, A., 1994. Neuronal differentiation signals are controlled by nerve growth factor receptor/Trk binding sites for SHC and PLC gamma. *EMBO J.* 13, 1585–1590.
- Olgart Höglund, C., de Blay, F., Oster, J.P., 2002. Nerve growth factor levels and localisation in human asthmatic bronchi. *Eur. Respir. J.* 20, 1110–1116.
- Penido, C., Costa, K.A., Costa, M.F., Pereira, J.F., Siani, A.C., Henriques, M.G., 2006. Inhibition of allergen-induced eosinophil recruitment by natural tetranortriterpenoids is mediated by the suppression of IL-5, CCL11/eotaxin and NF-kappaB activation. *Int. Immunopharmacol.* 6, 109–121.
- Secor, E.R., Carson, W.F., Singh, A., Pensa, M., Guernsey, L.A., Schramm, C.M., Thrall, R.S., 2008. Oral bromelain attenuates inflammation in an ovalbumin-induced murine model of asthma. *Evis. Based Compl. Alt. Med.* 5, 61–69.
- Sniderhan, L.F., Stout, A., Lu, Y., Chao, M.V., Maggirwar, S.B., 2008. Ankyrin-rich membrane spanning protein plays a critical role in nuclear factor-kb signaling. *Mol. Cell. Neurosci.* 38, 404–416.
- Tillie-Leblond, I., Pugin, J., Marquette, C.H., Lamblin, C., Saulnier, F., Brichet, A., Wallaert, B., Tonnel, A.B., Gosset, P., 1999. Balance between pro-inflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. *Am. J. Respir. Crit. Care Med.* 159, 487–494.
- Vanacker, N.J., Palmans, E., Kips, J.C., Pauwels, R.A., 2001. Fluticasone inhibits but does not reverse allergen-induced structural airway changes. *Am. J. Respir. Crit. Care Med.* 163, 674–679.
- Virchow, J.C., Julius, P., Lommatzsch, M., Luttmann, W., Renz, H., Braun, A., 1998. Neurotrophins are increased in bronchoalveolar lavage fluid after segmental allergen provocation. *Am. J. Respir. Crit. Care Med.* 158, 2002–2005.
- Wills-Karp, M., Uchida, Y., Lee, J.Y., Jinot, J., Hirata, A., Hirata, F., 1993. Organ culture with proinflammatory cytokines reproduces impairment of the beta-adrenoceptor-mediated relaxation in tracheas of a guinea pig antigen model. *Am. J. Respir. Cell Mol. Biol.* 8, 153–159.
- Wright, J.G., Christman, J.W., 2003. The role of nuclear factor kappa B in the pathogenesis of pulmonary diseases: implications for therapy. *Am. J. Respir. Med.* 2, 211–219.
- Wu, S.H., Arévalo, J.C., Sarti, F., Tessarollo, L., Gan, W.B., Chao, M.V., 2009. Ankyrin repeat-rich membrane spanning/Kidins220 protein regulates dendritic branching and spine stability in vivo. *Dev. Neurobiol.* 69, 547–557.
- Xu, K.F., Vlahos, R., Messina, A., Bamford, T.L., Bertram, J.F., Stewart, A.G., 2002. Antigen-induced airway inflammation in the Brown Norway rat results in airway smooth muscle hyperplasia. *J. Appl. Physiol.* 93, 1833–1840.
- Yin, H., Jin, X.B., Gong, Q., Yang, H., Hu, L.Y., Gong, F.L., Zhu, J.Y., 2008. Fructose-1,6-diphosphate attenuates acute lung injury induced by lipopolysaccharide in mice. *Int. Immunopharmacol.* 8, 1842–1847.
- Ying, S., Zhang, G., Gu, S., Zhao, J., 2006. How much do we know about atopic asthma: where are we now? *Cell Mol. Immunol.* 3, 321–332.
- Yoshinaka, T., Nishii, K., Yamada, K., Sawada, H., Nishiwaki, E., Smith, K., Yoshino, K., Ishiguro, H., Higashiyama, S., 2002. Identification and characterization of novel mouse and human ADAM33 with potential metalloprotease activity. *Gene* 282, 227–236.
- Zhu, M.M., Zhou, Q.H., Zhu, M.H., Rong, H.B., Xu, Y.M., Qian, Y.N., Fu, C.Z., 2007. Effects of nebulized ketamine on allergen-induced airway hyperresponsiveness and inflammation in actively sensitized Brown-Norway rats. *J. Inflamm.* 4, 4–10.
- Zimmermann, N., Hershey, G.K., Foster, P.S., Rothenberg, M.E., 2003. Chemokines in asthma: cooperative interaction between chemokines and IL-13. *J. Allergy Clin. Immunol.* 111, 227–242.