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House dust mite allergen Der f 2 induces *interleukin-13* expression by activating the PI3K/Akt pathway

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Abstract House dust mites (HDMs) are a common cause of allergic asthma. The group 2 allergen from *Dermatophagoides farinae*, Der f 2, is one of the major HDM allergens. Elevated Der f 2 immunoglobulin E (IgE) levels are observed in most of the allergic patients. *Interleukin-13* (*IL-13*), a gene associated with asthma pathology, was induced by Der f 2 in BEAS-2B human airway epithelial cells; however, the signaling pathways associated with Der f 2 are not fully understood. In this study, we identified a role of the phosphatidylinositol-3-kinase (PI3K)/Akt pathway, a well-known potential target for anti-asthma drugs, in the *IL-13* induction by Der f 2. First, Der f 2 activated the PI3K/Akt pathway, which subsequently activated the nuclear factor-kappa B (NF-κB) pathway and induced *IL-13* expression in BEAS-2B cells. Treatment with the PI3K inhibitor LY294002 abolished Der f 2-induced activation of Akt and NF-κB and the expression of *IL-13*. Furthermore, Der f 2-induced activation of the PI3K/Akt and NF-κB pathways, expression of *IL-13*, and the blockade of these effects with a PI3K inhibitor were confirmed in the lungs of mice that were intranasally exposed to Der f 2. Taken together, these results indicate that the PI3K/Akt pathway regulates Der f 2-induced *IL-13* expression via activation of the NF-κB pathway.

Keywords Der f 2 · IL-13 · PI3K · NF-κβ · Airway epithelial cells

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Introduction

Over the past 50 years, the prevalence of asthma has increased globally, particularly in developing countries. At present, an estimated 300 million people are affected by asthma worldwide, with an expected increase to 400 million by 2025 [1]. Previously, asthma was considered as a disease of bronchoconstriction and was primarily treated with bronchodilators. Recent studies show that asthma is an inflammatory disease of the airways in which T helper type 2 (Th2) cells and related cytokines interleukin-4 (IL-4), IL-5, and IL-13 are critical to asthma pathobiology [2, 3].

Fifty to eighty percent of asthmatics are typically allergic to house dust mites (HDMs), although this percentage depends on geographic location [4]. *Dermatophagoides pteronyssinus (Der p)* and *Dermatophagoides farinae (Der f)* are the most prevalent HDM species, and their associated allergens are common causes of asthma [2]. HDM associated allergens induce the expression of IL-13 and IL-4 in mite-sensitive asthmatic basophils and the release of IL-8 in airway epithelial cells [5].

The mite group 2 allergen Der f 2 is one of the most important HDM allergens, as Der f 2 immunoglobulin E (IgE) levels are elevated in 70–80 % of allergic patients [6]; however, Der f 2-induced cytokine expression and the underlying intracellular signaling pathways are not yet understood. Der f 2 stimulates the phospholipase C gamma/

protein kinase C alpha/p38 mitogen-activated protein kinase pathway in BEAS-2B cells by activating phospholipase D1 (PLD1) [5]. While each of the Th2 cytokines participates in the overall immune response against environmental allergens, a substantial body of evidence highlights a pivotal role for IL-13 in the regulation of allergic response. According to initial studies in animal disease models, IL-13 can induce all features of allergic asthma independent of other Th2 cytokines. In addition, the importance of IL-13 in allergic diseases in humans is supported by a strong association between tissue IL-13 levels and genetic variants in the *IL-13* gene with asthma and related traits [7]. Because of previous studies supporting the importance of IL-13 in allergic disorders, the signaling pathways inducing IL-13 expression in allergic asthma have been studied both in vitro and in vivo asthma models.

The phosphatidylinositol-3-kinase (PI3K), a member of a large family of lipid and serine/threonine kinases, is a potential target for the development of novel therapies for asthma. PI3K contributes to asthma pathogenesis by controlling multiple signaling pathways that lead to cell growth, proliferation, survival, and migration [8]. The PI3K signaling pathways suppress Th2 cytokine secretion, eosinophil infiltration, mucus secretion, and airway hyperreactivity in murine asthma models [8]. The PI3K/Akt pathway has been shown to regulate the nuclear factor-kappa B (NF-κB) pathway in several asthma models [9, 10]. Akt activates IκB kinase (IKK), which in turn phosphorylates IκBα. This phosphorylation results in a proteasomal degradation of IκBα and release of NF-κB from IκBα that trapped NF-κB in the cytosol [11]. Free NF-κB translocates into the nucleus and acts as a transcription factor for pro-inflammatory genes, such as Th2 cytokines [12].

As the first line of defense against external stimuli, such as antigens or viruses, airway epithelial cells play an important role in asthma by expressing pattern-recognition receptors to trigger host defense response, by interacting with dendritic cells to promote antigen sensitization, and by secreting cytokines to recruit effector cells [3, 10]. Therefore, airway epithelial cells act as initiators, mediators, and supervisors in innate and adaptive immune responses and effectively bridge innate and adaptive immunity. Because of these essential functions, airway epithelial cells are considered good therapeutic targets for the discovery and development of anti-asthma drugs. In addition, understanding the signaling pathways regulated by allergen exposure in these cells may lead to new therapeutic strategies for the treatment of asthma [3].

Therefore, we examined the relationship between the PI3K/Akt pathway and Der f 2-induced responses in BEAS-2B, human airway epithelial cells. We found that exposure to the clinically relevant allergen, Der f 2, induced rapid activation of PI3K, leading to phosphorylation of Akt and the eventual activation of NF-κB. This activation ultimately led to *IL-13* expression. Inhibition of PI3K and Akt attenuated Der f 2-induced NF-κB transactivation and *IL-13* expression. Furthermore, we confirmed the activation of the PI3K/Akt pathway and the NF-κB pathway in the lungs of mice exposed to Der f 2. Inhibition of PI3K attenuated the activity of the NF-κB pathway and *IL-13* expression by Der f 2 in mouse lungs, suggesting that the PI3K/Akt pathway is required for Der f 2-induced *IL-13* expression.

Materials and methods

Cell culture, transfection, and drug treatment

BEAS-2B, a human airway epithelial cell line transformed with adenovirus 12-SV40 virus hybrid, has been described previously [5]. BEAS-2B cells were cultured in Dulbecco's modified Eagle's medium/F12 (Welgene) with 10 % fetal bovine serum (Gibco), 100 units/ml penicillin (Gibco), and 100 ng/ml streptomycin (Gibco) at 37 °C with 5 % CO_2 in humidified air. Plasmid transfections were performed with Lipofectamine (Invitrogen) according to the manufacturer's instructions. LY294002 and BAY 11-7085 were obtained from A.G. Scientific and Calbiochem, respectively. Drugs were administered at the indicated concentrations: LY294002 at 20 μM and BAY 11-7085 at 2 μM. Der f 2 was treated with the concentrations indicated in the figure legends.

Plasmids

The pCMV6-HA, pCMV6-wild-type (WT) Akt-HA, and pCMV6-dominant negative (DN) Akt-HA plasmids were obtained from J. K. Chung (Seoul National University, Seoul, South Korea). The NF-κB reporter plasmid was kindly provided by M. B. Hershenson (University of Michigan, Michigan, USA). The pCMV-β-galactosidase (β-gal) reporter plasmid was obtained from Clontech.

Reverse transcription polymerase chain reaction (RT-PCR)

Total cellular and tissue RNA was isolated using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. For the reverse transcriptase reaction, 2 μg of total RNA (cellular) or 10 μg of total RNA (lung tissue) was mixed with an oligo$(dT)_{16}$ primer and Maloney murine leukemia virus reverse transcriptase (Invitrogen), and the mixture was incubated for 60 min at 37 °C. The transcribed products were mixed with each primer set and premix (SGBIO) and amplified. The primer sequences were as follows: human *IL-13* sense (5′-GAGTGTGTTTG

TCACCGTTG-3′) and antisense (5′-TACTCGTTGGTCG AGAGCTG-3′) to generate a PCR product of 275 bp, mouse *IL-13* sense (5′-CTCCCTCTGACCCTTAAGGAG-3′) and antisense (5′-GAAGGGGCCGTGGCGAAACAG-3′) to generate a PCR product of 308 bp, human *GAPDH* sense (5′-AAGGTCGGAGTCAACGGATTTGGT-3′) and antisense (5′-AGTGATGGCATGGACTGTGGTCAT-3′) to generate a PCR product of 533 bp, and mouse *GAPDH* sense (5′-ACCACAGTCCATGCCATCAC-3′) and antisense (5′-TCCACCACCCTGTTGCTGTA-3′) to generate a PCR product of 451 bp. The PCR products were analyzed in a 2 % agarose gel.

Western blot analysis

Cells were first lysed in radio immunoprecipitation assay (RIPA) buffer [150 mM NaCl; 10 mM Tris, pH 7.2; 0.1 % sodium dodecyl sulfate; 1 % Triton X-100; 1 % sodium deoxycholate; 5 mM ethylenediaminetetraacetic acid (EDTA)]. Proteins (15–30 μg) were subsequently loaded onto sodium dodecyl sulfate–polyacrylamide gels (10 %) and transferred to nitrocellulose membranes (Whatman). After blocking with 5 % dried skim milk for 2 h, the membrane was incubated with primary antibodies (anti-p-Akt, anti-p-p38, and anti-p-IκBα from Cell Signaling Technology, anti-α-tubulin from Calbiochem, and anti-HA (hemagglutinin) from Santa Cruz Technology). The blots were further incubated with horse radish peroxidase-conjugated anti-mouse (Cell Signaling Technology) or anti-rabbit (Bio-Rad) secondary antibodies.

NF-κB transactivation

Cells were transfected with the NF-κB reporter and pCMV-β-gal reporter plasmid. Approximately 24 h after transfection, the cells were starved for 24 h by growing in the medium-depleted serum. The cells were treated with Der f 2 (10 μg/ml) for 24 h. Cells were harvested and lysed in the Reporter Lysis Buffer (Promega) according to the manufacturer's instructions. Luciferase activities were normalized to β-galactosidase activities, an internal control. The results are expressed as the means ± standard deviations (SD) from three independent experiments.

Animal experiment

All animal experiments were performed in accordance with the Korean Food and Drug Administration guidelines. Protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Yonsei Laboratory Animal Research Center. Six-week-old male BALB/c mice were purchased from Koatech. Der f 2 (50 μg/ml) was intranasally administered to each nostril, and LY294002 (7.5 mg/kg in 10 μl DMSO) was intranasally administered 2 h prior to Der f 2 administration. Six hours after Der f 2 challenge with or without LY294002 administration ($n = 5$), mouse lungs were obtained and fixed in 4 % paraformaldehyde (Duksan), and the tissue sections were stained with hematoxylin and eosin (H&E).

Immunohistochemistry

The 4-μm paraffin-embedded tissue sections were deparaffinized and rehydrated. For antigen retrieval, the slides were autoclaved in 10 mM sodium citrate buffer (pH 6.0, Sigma-Aldrich) for 15 min. Endogenous peroxidase was blocked with 0.345 % H_2O_2 (Samchun Chemicals) for 30 min, and sections were further blocked with 5 % bovine serum albumin in phosphate-buffered saline (PBS) for 30 min. For mouse primary antibody, mouse IgG was blocked by M.O.M mouse IgG blocking kit (Vector Laboratories). The sections were incubated with primary antibody overnight at 4 °C. The concentrations of the primary antibodies were as follows: p-Akt (1:100) and p-IκBα (1:100). Sections were incubated with biotinylated anti-mouse (Dako; 1:300) or biotinylated anti-rabbit (Dako; 1:300) secondary antibodies for 1 h at room temperature, followed by incubation with avidin–biotin complex solutions (Vector Laboratories; 1:500). The sections were stained with 3,3′-diaminobenzidine (DAB) staining solution (Dako) for 3–7 min and counterstained with Mayer's hematoxylin (Muto). The DAB-stained preparations were visualized with a general optical microscope (TE-2000, Nikon). All incubations were conducted in humid chambers. At least three fields per section were analyzed to establish reproducibility.

Statistical analysis

All data are expressed as the mean ± SD, and sample numbers are represented at each figure legend. Statistical analyses were performed using Student's *t* test. Results are representative examples of three independent experiments. Significance was accepted at $P < 0.05$.

Results

Der f 2 induces *IL-13* expression and activates Akt in human bronchial epithelial cells

Previous studies reported that Der f 2 activated the mitogen-activated protein kinase/p38 and PLD1 signaling pathways and that these signaling pathways were involved in *IL-13* mRNA expression ([5]; Fig. 1a). Following Der f 2 treatment to BEAS-2B cells, *IL-13* mRNA expression was increased at 15 min and sustained up to 60 min (Fig. 1a). In addition, Der f 2 induced *IL-13* mRNA expression in a dose-dependent manner (Fig. 1b). Since the

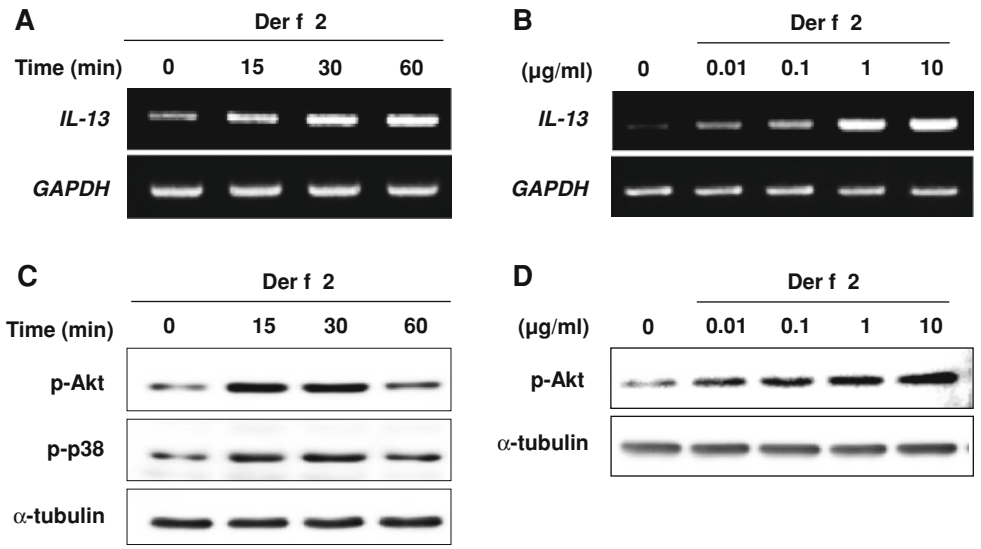

Fig. 1 Effects of Der f 2 on induction of *IL-13* and activation of Akt in BEAS-2B cells. BEAS-2B cells were incubated with Der f 2 (10 μg/ml) for the indicated time periods. *IL-13* mRNA expression levels were detected by RT-PCR, and phosphorylated Akt and p38 were detected by immunoblotting of whole cell lysates (WCLs) with the indicated antibodies (**a, c**). BEAS-2B cells were treated with the indicated concentrations of Der f 2 for 15 min. *IL-13* mRNA expression levels were determined using RT-PCR, and phosphorylated Akt was detected by immunoblotting with the indicated antibody (**b, d**)

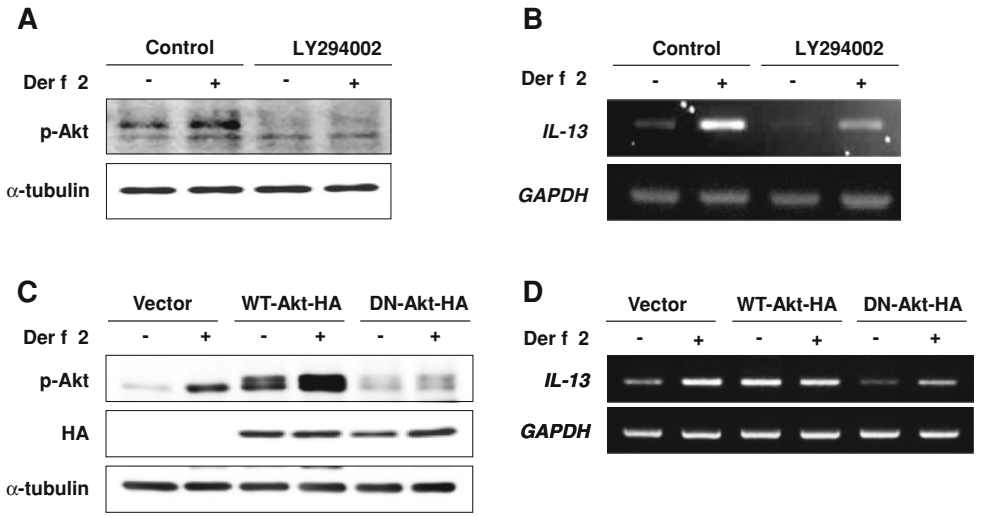

Fig. 2 Effects of chemical or genetic inhibition of the PI3K/Akt pathway on induction of *IL-13* in BEAS-2B cells. BEAS-2B cells were pretreated with LY294002 (20 μM for 1 h) and stimulated with Der f 2 (10 μg/ml for 15 min). Untreated cells of the control group were pretreated with dimethyl sulfoxide (DMSO) (**a, b**). **a** WCLs were subjected to immunoblotting using the indicated antibody. **b** *IL-13* mRNA expression was detected by RT-PCR. BEAS-2B cells were transfected with pCMV-HA, WT-Akt-HA, and DN-Akt-HA plasmids for 24 h, and cells were treated with Der f 2 (10 μg/ml for 15 min) (**c, d**). **c** Phosphorylation of Akt and HA levels was detected by immunoblotting using the indicated antibodies. **d** *IL-13* mRNA levels were detected by RT-PCR

PI3K/Akt pathway induces Th2 cytokines in mouse asthma model induced by ovalbumin [8, 13], activation of the PI3K/Akt pathway by Der f 2 was investigated. Within 15 min, Der f 2 increased phosphorylation of Akt as well as that of p38, which is known to be activated by Der f 2 [5], and the phosphorylation levels were sustained for up to 30 min (Fig. 1c). Furthermore, the activation of Akt was increased by Der f 2 in a dose-dependent manner (Fig. 1d).

Der f 2 induces *IL-13* expression via the PI3K/Akt pathway

To investigate whether the PI3K/Akt signaling pathways are associated with Der f 2-induced *IL-13* expression, we measured the effect of LY294002, a chemical inhibitor of PI3K, on Der f 2-induced *IL-13* expression. First, we showed that Akt activity was inhibited by LY294002 pre-

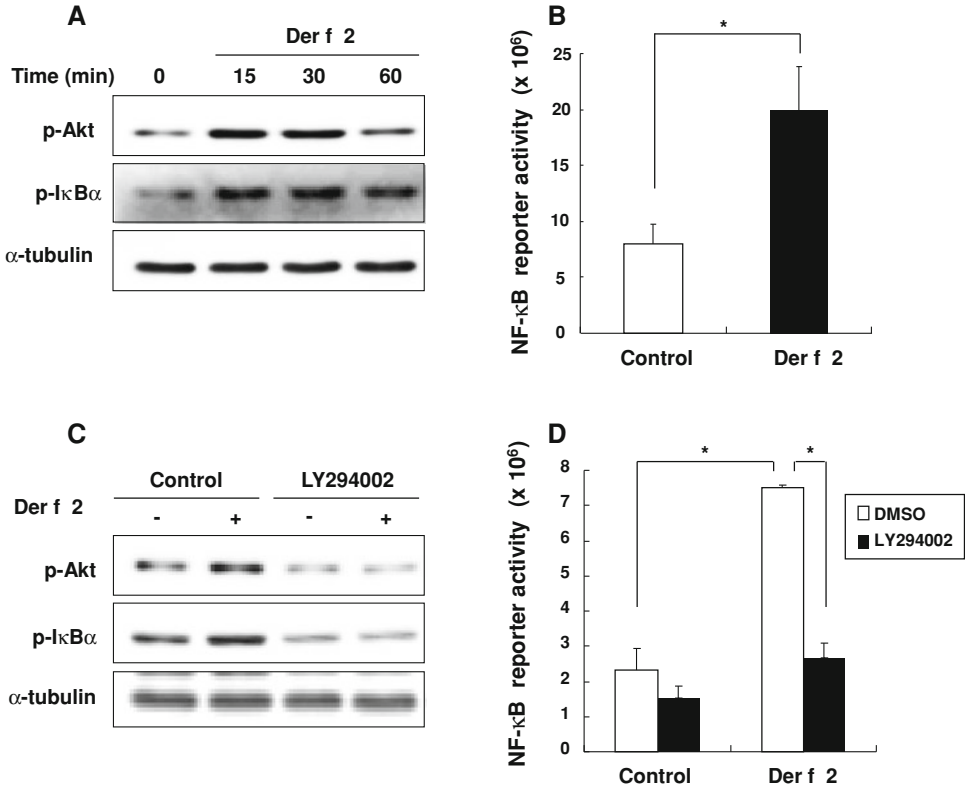

Fig. 3 Effect of LY294002 on Der f 2-induced activation of the NF-κB pathway. **a** BEAS-2B cells were incubated with Der f 2 (10 μg/ml) for 15 min, and the levels of phosphorylated Akt and IκBα were analyzed by immunoblotting with specific antibodies. **b** Cells were transfected with the NF-κB reporter plasmid and treated with Der f 2 (10 μg/ml for 24 h), and then the NF-κB reporter activity in these cells was measured. **c** BEAS-2B cells were pretreated with LY294002 (20 μM for 1 h) and stimulated with Der f 2 (10 μg/ml for 15 min). WCLs were subjected to immunoblotting using indicated antibodies. **d** BEAS-2B cells were transfected with the NF-κB reporter plasmid, pretreated with LY294002 (20 μM for 1 h), and treated with Der f 2 (10 μg/ml for 24 h), and then the NF-κB reporter activity in these cells was measured. Results are representative examples of three independent experiments. $*P < 0.05$

treatment, demonstrating that, in the context of Der f 2 exposure, Akt functions downstream of PI3K (Fig. 2a). In BEAS-2B cells, LY294002 attenuated Der f 2-induced *IL-13* mRNA expression (Fig. 2b). We also examined whether Akt was required for Der f 2-induced responses by measuring the effects of a dominant negative form of Akt (DN-Akt). Overexpression of wild-type Akt (WT-Akt) increased the levels of phosphorylated Akt in both Der f 2-treated and non-treated cells; however, the expression of DN-Akt decreased the Der f 2-induced phosphorylation of Akt (Fig. 2c). Overexpression of WT-Akt increased *IL-13* mRNA expression to the levels induced by treatment with Der f 2, but this level was not further increased by treatment of Der f 2 in cells that overexpressed WT-Akt (Fig. 2d). The Der f 2-induced *IL-13* mRNA expression, however, was decreased by overexpression of DN-Akt (Fig. 2d). Therefore, in the context of Der f 2 exposure to BEAS-2B cells, Akt functions downstream of PI3K, and the PI3K/Akt pathway is required for Der f 2-induced *IL-13* mRNA expression.

Der f 2 activates the NF-κB pathway via the PI3K/Akt pathway

To investigate whether the Der f 2-induced activation of the PI3K/Akt pathway regulates other signaling pathways to promote *IL-13* expression, we examined the NF-κB pathway, which has been shown to be a target of Akt in some asthma studies [9]. We monitored activation of NF-κB by detecting $Ser^{32/36}$ phosphorylation of IκBα following Der f 2 treatment. Der f 2 increased IκBα phosphorylation concomitant with the phosphorylation of Akt (Fig. 3a). To determine whether Der f 2 induces NF-κB transactivation, BEAS-2B cells were transfected with a vector harboring NF-κB-responsive promoter elements fused to the luciferase gene (NF-κB-TATA-Luc) and treated with Der f 2. Der f 2 increased NF-κB reporter activity (Fig. 3b). The increased phosphorylation of Akt and IκBα as well as the increased NF-κB reporter activity following Der f 2 exposure was abolished by LY294002 (Fig. 3c, d). Together, these results demonstrate that Der f

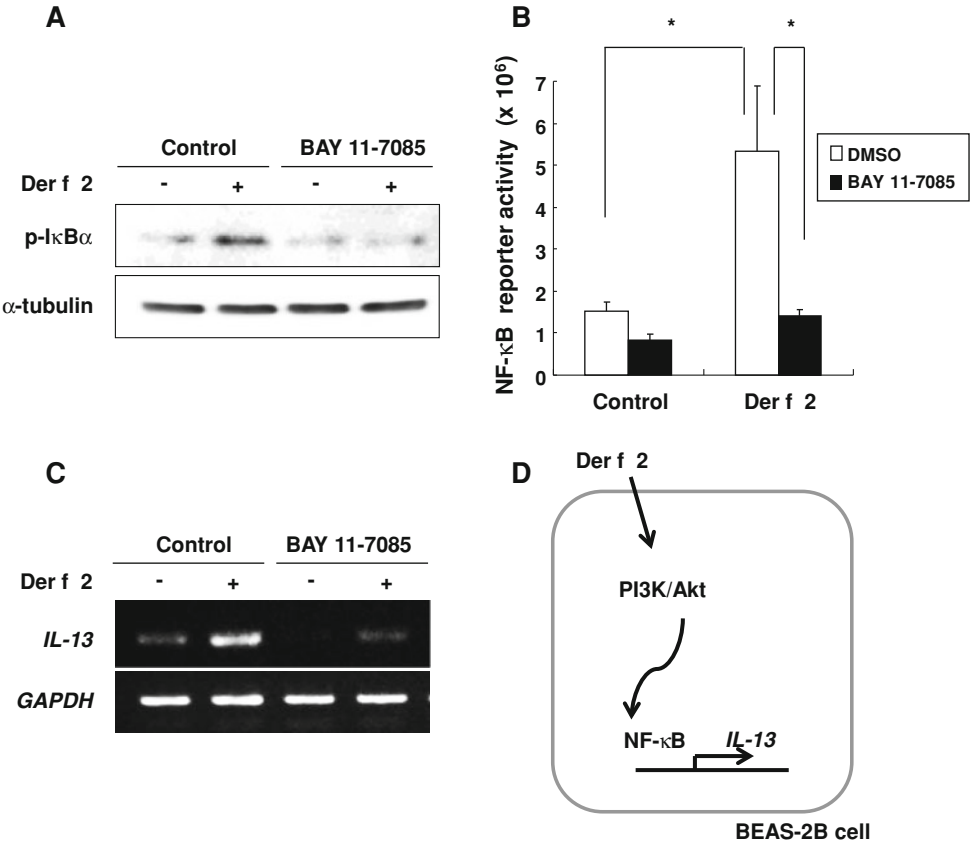

Fig. 4 Effect of BAY 11-7085, an inhibitor of NF-κB, on Der f 2-induced expression of *IL-13*. **a** BEAS-2B cells were pretreated with LY294002 (20 μM for 1 h) and stimulated with Der f 2 (10 μg/ml for 15 min). After lysis, phosphorylation of IκBα was analyzed by immunoblotting using the indicated antibody. **b** BEAS-2B cells were transfected with the NF-κB reporter plasmid, pretreated with BAY 11-7085 (2 μM for 1 h), and treated with Der f 2 (10 μg/ml for 24 h), and then the NF-κB reporter activity in these cells was measured. Results are representative examples of three independent experiments. $*P < 0.05$. **c** BEAS-2B cells were pretreated with BAY 11-7085 (2 μM for 1 h) and stimulated with Der f 2 (10 μg/ml for 15 min). *IL-13* mRNA levels were determined by RT-PCR. **d** The mechanism by which Der f 2 induces expression of *IL-13* in BEAS-2B cells is shown

2 activates the NF-κB pathway via the activation of the PI3K/Akt pathway.

NF-κB is required for Der f 2-induced *IL-13* expression

Since Der f 2-induced activation of the PI3K/Akt pathway upregulated the NF-κB signaling pathway, the requirement of NF-κB for Der f 2-induced *IL-13* expression was studied. In BEAS-2B cells, the IKK inhibitor, BAY 11-7085, effectively inhibited the phosphorylation of IκBα by Der f 2 (Fig. 4a). The effects of IKK inhibition on Der f 2-induced NF-κB transactivation were tested using the NF-κB reporter system. Treatment with BAY 11-7085 not only inhibited the basal level of NF-κB transactivation but also reduced Der f 2-induced NF-κB transactivation to the basal level in BEAS-2B cells (Fig. 4b). The inhibition of Der f 2-induced NF-κB pathway activation by BAY 11-7085 resulted in decreased Der f 2-induced *IL-13* mRNA expression (Fig. 4c). Together, these results demonstrate that Der f 2 regulates NF-κB-dependent gene regulation and that NF-κB signaling is critical for Der f 2-induced *IL-13* expression (Fig. 4d).

Der f 2 induces *IL-13* expression via the PI3K/Akt/NF-κB pathway in vivo

To further assess whether Der f 2 induces *IL-13* expression via the PI3K/Akt/NF-κB pathway in vivo, mice were intranasally exposed to LY294002 2 h prior to Der f 2 exposure, and lung tissue samples were obtained 6 h following the challenge in order to determine pathway activity. H&E-stained tissues obtained from Der f 2-challenged mice did not exhibit any overt pathological changes, such as eosinophil infiltration (Fig. 5a). The *IL-13* mRNA levels in the lung tissue lysates were increased by Der f 2, and this increase was inhibited by the administration of LY294002 (Fig. 5b). Phosphorylation of Akt and IκBα was also detected in the lung tissue lysates, and this

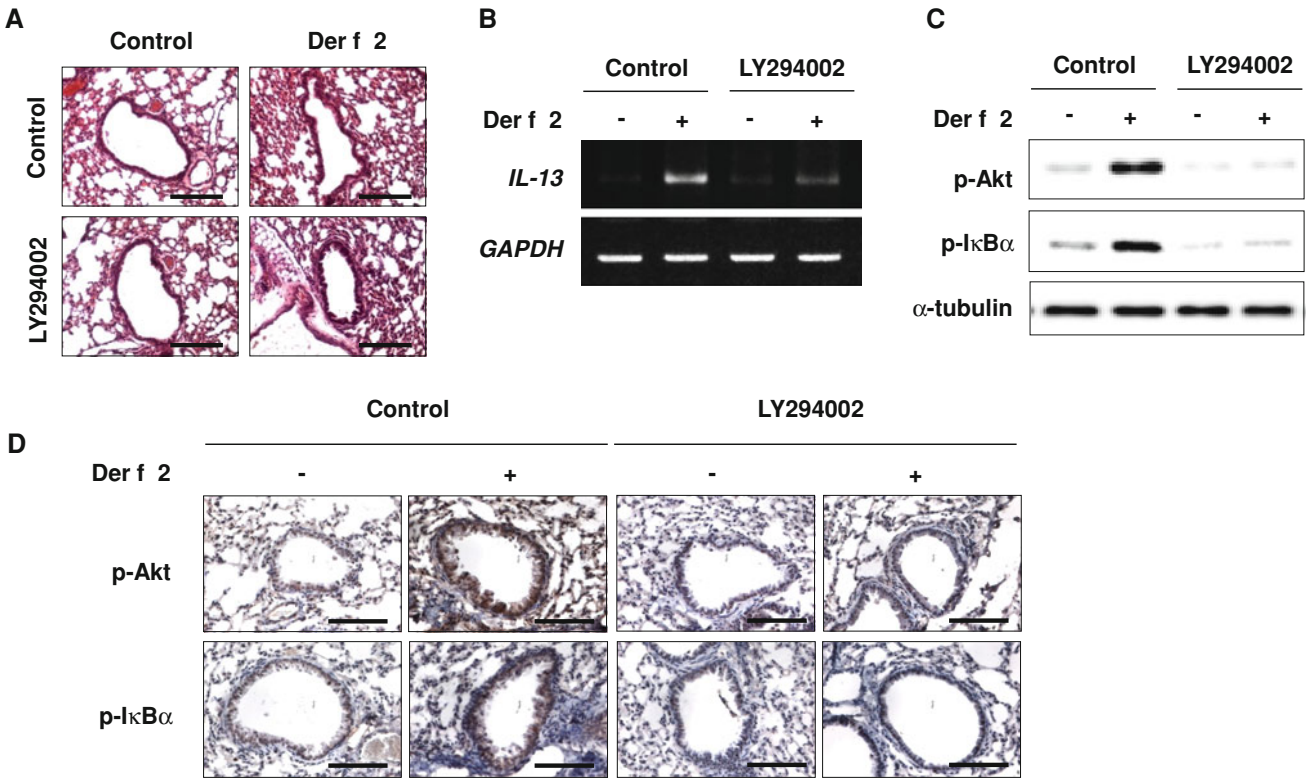

Fig. 5 Der f 2 induces *IL-13* expression via the PI3K/Akt/NF-κB pathway in vivo. BALB/c mice were challenged with Der f 2 or PBS with or without pre-administration with DMSO or LY294002 (7.5 mg/kg in 10 μl DMSO for 2 h). Six hours after the challenge, lung tissue samples were collected. **a** H&E staining of the lung tissue sections. **b** *IL-13* mRNA expression was detected by RT-PCR. **c** Phosphorylated Akt and IκBα were detected by immunoblotting with indicated antibodies. **d** Immunohistochemistry using 3,3′-diaminobenzidine (DAB) with the indicated antibodies detected phosphorylated Akt and IκBα levels in mouse lung tissues. The histological changes to the lung tissue were examined (*scale bar* 100 μm; **a, d**)

phosphorylation was increased in the Der f 2-challenged group compared to the PBS-challenged group. Pre-administration of LY294002 inhibited this Der f 2-induced phosphorylation of Akt and IκBα (Fig. 5c). Furthermore, Der f 2 administration increased the phosphorylation of Akt and IκBα especially in the bronchial epithelial cells in the lung tissue sections, and these increases were inhibited by pre-administration of LY294002 (Fig. 5d). These data demonstrate that Der f 2 induces *IL-13* expression via the PI3K/Akt/NF-κB pathway in the lung in a mouse model.

Discussion

HDMs trigger the majority of allergic asthma, and their associated allergens, Der p and Der f, induce the expression and production of inflammatory cytokines that are considered as key to immunity-related diseases. Der f 2 is a clinically relevant allergen, which sensitizes most of the allergic patients [2, 6]. Emerging studies have focused on alleviation of Der f 2-induced asthma in vivo by loading chitosan nanoparticles or by constructing Fcγ-Der f 2 fusion proteins; however, little is known about the mechanisms by which Der f 2 exerts allergic inflammation [6, 14]. In human bronchial epithelial cells, Der f 2 induces *IL-13* expression via the PLD1-regulated signaling pathways [5]; however, extensive studies have demonstrated the involvement of the PI3K/Akt pathway in allergic asthma both in vitro and in vivo, suggesting the PI3K/Akt pathway as a potential target for asthma therapy. Inhibition of PI3K effectively reduced ovalbumin-induced asthma-related phenotypes, such as pulmonary eosinophilia, mucus hypersecretion, and airway hyperreactivity in a mouse asthma model [15]. Furthermore, in allergen-induced asthma models, the PI3K/Akt pathway induced *IL-13* mRNA expression and protein secretion. Despite the importance of the PI3K/Akt pathway in allergic responses, the involvement of this pathway in the Der f 2-induced responses has not been studied. Therefore, in this study, we investigated the activation of the PI3K/Akt pathway by Der f 2. In BEAS-2B cells, Der f 2 induced expression of *IL-13* in a time- and dose-dependent manner and activated the PI3K/Akt pathway within a short period of

time. We showed that inhibition of the PI3K/Akt pathway, either by the PI3K inhibitor LY294002 or by overexpression of a dominant negative form of Akt, attenuates Der f 2-induced expression of *IL-13*, although this inhibition does not completely block the induction of *IL-13* expression, possibly due to the involvement of other signaling pathways in the Der f 2-induced responses. Moreover, we demonstrated that the NF-κB pathway is activated by treatment with Der f 2 and that activation of the PI3K/Akt pathway is required for Der f 2-induced NF-κB transactivation. In allergic asthma models, NF-κB has been shown to be required for allergen-induced expression of pro-inflammatory molecules, such as IL-4, IL-5, IL-13, IL-6, and IL-8 [12]. Here, we extended this result by showing that phosphorylation of IκBα by IKK is required for Der f 2-induced expression of *IL-13*, suggesting that the NF-κB-inducing kinase is the upstream activator of IκBα in this context. Furthermore, our results indicated that in human bronchial epithelial cells, which serve as the first line of defense to foreign agents in the lung, Der f 2 induces the expression of *IL-13* by activating the PI3K/Akt/NF-κB pathway. Der f 2 also induced *IL-13* expression via the same mechanism in vivo in a mouse model. Der f 2 significantly increased the phosphorylation levels of Akt and IκBα in the mouse lung tissue, especially in the mouse bronchial epithelial cells, and these Der f 2-induced effects were inhibited by pre-administration of LY294002. In conclusion, this study provides strong evidence that Der f 2-induced *IL-13* expression is mediated by the activation of the PI3K/Akt/NF-κB pathway in human bronchial epithelial cells and in normal mouse lungs. Identification of the PI3K/Akt pathway as a route for Der f 2-induced *IL-13* expression implicates this pathway as a novel therapeutic target for treatment of asthma related to HDM.

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Conflict of interest The authors have no conflicting financial interest.

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