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β-arrestin2 Stimulates Interleukin-17 Production and Expression of $CD4^+$ T Lymphocytes in a Murine Asthma Model

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ABSTRACT

Allergic asthma is a complex and chronic inflammatory airway disease. Interleukin-17 is a pro-inflammatory cytokine which plays critical role in the pathogenesis of allergic asthma. It has been reported that β-arrestin2 regulated the development of allergic asthma at a proximal step in the inflammatory cascade. In this study, the influence of β-arrestin2 on Interleukin-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model was investigated.

Splenic $CD4^+$ T lymphocytes from wild-type mice and those from a murine asthma model were purified. $CD4^+$ T lymphocytes from a murine asthma model were transfected with siRNAs targeting the β-arrestin2 or were pretreated with the ERK1/2 inhibitor, PD98059. After stimulation, the protein expression of β-arrestin2、phosphorylated-ERK1/2 and IL-17 were detection by Western blot; the mRNA expression of IL-17 were detected by real-time PCR; the accumulation of IL-17 in supernatants were detected by ELISA.

We found that β-arrestin2、phosphorylated-ERK1/2 and IL-17 expression in $CD4^+$ T lymphocytes from a murine asthma model was increased compared with those from wild-type mice($p<0.01$). Treatment of $CD4^+$ T lymphocytes with siRNAs targeting the β-arrestin2 down-regulated phosphorylated- ERK 1/2 and IL-17 expression ($p < 0.01$). PD98059 decreased IL-17 production and expression in $CD4^+$ T lymphocytes in a murine asthma model ($p < 0.05$).

We conclude that β-arrestin2 stimulated IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model. The effect was partly mediated by ERK 1/2 activation. Targeting β-arrestin2 biological activity could be a valid therapeutic approach for the treatment of allergic asthma.

Keywords: Asthma; β-arrestin2; $CD4^+$ T Lymphocytes; Extracellular Signal-regulated Kinase 1/2; Interleukin-17

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INTRODUCTION

Allergic asthma is a complex and chronic inflammatory disease characterized by obstruction airway due to inflammation and hyper responsiveness, which is at least partially reversible. Many cells and cellular elements such as mast cells, eosinophils, T lymphocytes, macrophages, neutrophils and epithelial cells play a role in the pathogenesis of asthma. Among these inflammatory cells, T lymphocytes play major roles.

Naïve $CD4^+$ helper T lymphocytes can develop into at least four types of committed helper T cells, namely T helper 1 (Th1), Th2, Th17 and regulatory T cells (Tregs) depending on the cytokine milieu.Th17 is characterized by the secretion of Interleukin-17(IL-17). It has been recognized as a novel pro-inflammatory $CD4^+$ T effector cell.[1, 2] TGF-β and IL-6 are essential for Th17 cell differentiation.[3, 4] IL-17 produced by Th17 has critical role in immunity to extracellular bacteria and the pathogenesis of several autoimmune disorders.[5, 6]

Recent studies have suggested that IL-17 was involved in the pathogenesis of allergic asthma. Several lines of evidence suggested that IL-17 could trigger allergic asthma by stimulating innate immunity and induce neutrophilic airway inflammation in murine asthma models.[7-9] It has also been shown that IL-17 is expressed in the airways of asthmatic patients and its expression is correlated with the severity of asthma.[10-12] IL-17 has also been shown to stimulate bronchial fibroblasts, epithelial cells and smooth muscle cells and induce the expression of a variety of cytokines and chemokines, which are important for granulopoiesis and neutrophil recruitment.[13] Moreover, it has recently been shown that IL-17-mediated neutrophilic airway inflammation is steroid-resistant. [14] These findings suggest that IL-17 plays crucial roles in causing airway inflammation in asthma and is paralleled with the severity of asthma.

β-arrestins, members of the arrestin family of proteins, designated β-arrestin1 and β-arrestin2,are ubiquitously expressed in most tissues and play an important role in regulating signal transduction by numerous G-protein-coupled receptors(GPCRs).[15] They are initially known as negative regulators of GPCR-mediated signaling. However, new roles of β-arrestins in receptor trafficking and signaling have been discovered in recent years.[16, 17]

We now know that they also serve as scaffolds and adapters in receptor endocytosis and signal transduction. They recruit endocytic proteins and a variety of signaling molecules to the receptors, thus connecting GPCRs to various pathways, such as mitogen-activated protein kinase (MAPK) cascades including extracellular signal-regulated kinase1/2 (ERK1/2), Jun N-terminal kinase (JNK), one of the p38 kinases, and Src family kinases.[17] One study has found the result that the proteomic expressions of β-arrestins were increased in T-lymphocytes of asthmatic patients compared to healthy persons.[18] In addition in an animal study, it was suggested that β-arrestin2 regulates the development of allergic asthma by regulation of T-cell migration. Moreover, it was also proposed that β-arrestin2 might make a contribution by regulation of other T cell functions or modulation of cells other than lymphocytes.[19]

Recently one study has demonstrated that β-arrestin2 positively mediated IL-6 production which is very important for the IL-17 production and this was mediated, in part, by the ERK 1/2 signaling pathway.[20] Furthermore, it was suggested that IL-17 production and expression in murine lymph node cells can be regulated by utilization of MAPK (ERK1/2, p38 and JNK) signaling.[21] So, we propose a hypothesis that β-arrestin2 may regulate IL-17 production and expression partly through ERK 1/2 signaling pathway in allergic asthma. The aim of this study was to determine the contribution of β-arrestin2 to IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model.

MATERIALS AND METHODS

Animal

Female BALB/c mice were obtained from Slaikejingda Laboratory (Changsha, China) at 6-8 weeks of age, weighed between 18-22g. All mice were maintained under pathogen-free conditions. They were raised and kept on an OV albumin (OVA) free diet. All experimental procedures were approved by Animal Care and Use Committee of the Central South University, and were conducted in accordance with the guidelines of the Central South University on Animal Care.

OVA-Induced Asthma Model

The asthma model utilized a 3-week OVA sensitization and challenge protocol, as described previously.[22] Briefly, the mice from asthma model group were sensitized using intraperitoneal injection of 25 μg chicken OVA (endotoxin-free, Grade V, Sigma Chemical Company) in 0.2ml of PBS, and mixed with 1 mg aluminum hydroxide gel (Serva Electrophoresis, Heidelberg, Germany) on days 0 and 7. The mice from wild-type (WT) group were given OVA using intraperitoneal injection of 0.2ml PBS. From day 14, the mice from asthma model group were challenged 25 min/day with nebulized 6% chicken OVA (endotoxin-free, Grade V, Sigma Chemical Company) in PBS up to day 20, using a Boehringer-Ingelheim nebulizer. The mice from wild-type (WT) group were challenged 25 min/day with PBS alone. On day 21, pulmonary function testing and methacholine challenge were performed prior to tissue harvesting and euthanasia.

Cell Counts in the Bronchoalveolar Lavage Fluid

Twenty-four hours after the final OVA or PBS challenge, bronchoalveolar lavage (BAL) was performed immediately after bleeding of the mice by three injections of 0.5 ml saline (37°C) through a tracheal cannula into the lung. Cells in the BAL were centrifuged and resuspended in cold PBS. The total number of cells in the BAL was determined using a counting-chamber. For differential BAL cell counts, cytospin preparations were made (1500rpm, 5min, 4°C, Eppendorf Centrifuge Configurator).Next, cells were fixed and stained with Wright's Giemsa stain.200 cells were counted under light microscopy .

Measurement of Airway Responsiveness

Twenty-four hours after the final OVA or PBS challenge, airway responsiveness in mice was determined using an animal airway resistance recorder by Buxco pulmonary function Apparatus referred to in previous study.[23] Briefly after anesthesia with 5% chloral hydrate 500 mg / kg, mice were implemented with tracheal intubation and connecting with small animal respirator. First record the basis of airway resistance values for 1 min; subsequently determined airway resistance changes after different concentration of methacholine (Mch) aerosol challenge. Each atomization costed 1 min and record 3 min. Concentration of inspired Mch was from low to high as follows: 0.39, 0.78, 1.56, 3.12 mg/ml. Airway resistance (Rl) and dynamic lung compliance (Cdyn) were obtained by measuring mouse airway flow and pressure.

Magnetic Activated Cell Sorting (MACS)

$CD4^+$ T lymphocytes from spleen cell suspensions were purified by using a magnetic-activated cell sorter system according to manufacturer's instructions (> 95% purity, Miltenyi Biotec). Briefly, spleen cells were incubated with RBC lysate 3-5 times volume of the cells (Beyotime Institute of Biotechnology) for 2 min at room temperature. After two washes, cells were incubated with streptavidin microbeads (100 μl/1 x 10^8 cells) at 1 x 10^8 /ml for 15 min at 4°C in MACS buffer. After two additional washes, bead-bound cells were isolated using a separation column placed in a strong magnetic field, and the positive fraction containing the $CD4^+$ T lymphocytes was harvested. The number of viable $CD4^+$ T lymphocytes was determined by trypan blue exclusion.

RNA Interference

Small interfering RNA sequences (siRNAs) targeting the mouse β-arrestin2 gene were designed and chemically synthesized by Shanghai GenePharma Co,Ltd. The following siRNA were used: β-arrestin2-1123: 5-CGAAUUCGAUACCAACUAUTT-3, 5-AUAGUUGGUAUCGAAUUCGAT-3. $CD4^+$ T lymphocytes were transfected with siRNAs (see above) using the Lipofectamine 2000 (Invitrogen) according to the modified manufacturer's instructions. Briefly, siRNAs were diluted by DEPC water (1 OD/150μl). 7.5μl siRNAs and 250μl Opti-MEM were mixed and incubated for 20min at Room temperature. 5μl lipofectamine 2000 was diluted by 200μl opti-MEM. Then the siRNA mixtures and lipofectamine 2000 were mixed by inversion and incubated for 5 min at room temperature. The entire transfection mixture was added to cell culture flasks containing 2.5×10^6 cells (2.5ml) .Following a incubation for 6 hours we determined the transfection efficiency by Fluorescence microscope. Cells were incubated for 24h at 37°C, and the medium was replaced with normal (serum-containing) growth medium. After additional incubation for 24h, silence efficiency was examined by westernblot.

Cell Culture

Splenic $CD4^+$ T lymphocytes with or without RNA interference were pretreated with or without

PD98059(Sigma Chemical Company)(5μM) [24] for 2 h followed by stimulated with Phorbol-12-myristate-13-acetate(PMA) (100ng/ml)[25] and concanavalin A(con A) (5μg/ml)[25] for 24 h(1×10^6 cells/ml) at 37°C in 5% CO_2,95% air. Next, the supernatant and cell were collected for analysis.

ELISA

Supernatants were harvested from $CD4^+$ T lymphocytes culture solution. IL-17 were quantified using the commercially available enzyme-linked immunosorbent assay (ELISA) kits for murine IL-17(R&D systems Inc, sensitivity <2 pg/ml) according to the manufacturer's instructions. Samples were tested at least in duplicate. Values for duplicate wells were calculated using a standard curve which was obtained on the basis of known concentrations of applied recombinant cytokines. Results are shown as mean ± standard deviation (SD) of three experiments.

Real-time PCR

Oligonucleotide primers for the target genes were designed by Primer Express TM Version 2.0 software (PE Applied Biosystems, Inc.). β-actin is chosen as an endogenous control and sequences of the primer sets used for RT-PCR verification are as follows:IL-17,forward:5-CTGGGAAGCTCAGTGCCGCC-3, reverse: 5-CAGGTGCAGCCCACACCCAC-3; β-actin forward:5-TACAGCTTCACCACCACAGC-3, reverse:5-AAGGA AGGCTGGAAAAGAGC-3. Total RNA was extracted from $CD4^+$ T lymphocytes using Trizol reagents (Invitrogen, USA). RNA (2μg) was reverse transcribed to generate cDNA using RevertAid™ H Minus First Strand cDNA Synthesis Kit (MBI Fermentas).

Real-time PCR reactions were performed by using the SYBR Green PCR Master Mix protocol (ABI, 4309155). PCR was performed according to the following conditions: initial denaturation at 95 ºC for 5 min, 40 cycles of denaturation at 94 ºC for 20s, annealing at 57 ºC for 20s and extension at 72 ºC for 20s, followed by a final extension at 72 ºC for 5 min. Three replicates were run for each gene for each sample .The comparative threshold cycle (Ct) value method was used to analyze the relative gene expression data.

Western Blot

Whole cell lysates were prepared from cells with ice-cold lysis buffer (20 mM Tris PH7.5, 1% TritonX-100, 150 mM NaCl, 2.5 mM sodium pyrophosphate, 1 mM EDTA, 1%Na3VO4, 0.5 μg/ml leupeptin) (Beyotime, China) supplemented with 1 mM PMSF. Protein concentration was measured with a BCA Protein Assay Kit (Beyotime, China). Lysates were added to Laemmli sample buffer and boiled for 4 min. Protein from each sample was subsequently subjected to a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto a nitrocellulose membrane (Pierce, USA). Non-specific binding was blocked with 5% non-fat milk in TBST(50 mM Tris-HCl, 150 mM NaCl, pH7.5, 0.1% Tween 20) for 2 h at 37 ºC.

After washes with TBST, membranes were incubated with a rabbit polyclonal antibody anti-phospho-p44/p42 MAP Kinase primary antibody(1:500 dilution)(Santa Cruz Biotechnology, USA) to detect phosphorylated-ERK 1/2(p- ERK 1/2), a rabbit polyclonal antibody anti-β-arrestin2 primary antibody(1:500 dilution)(Santa Cruz Biotechnology, USA) to detect β-arrestin2 expression and a rabbit polyclonal antibody anti-IL-17 primary antibody (1:500 dilution)(Santa Cruz Biotechnology, USA) to detect IL-17 expression. The blots were incubated overnight at 4 ºC and then with corresponding HRP-conjugated secondary antibodies (1:5000)(Santa Cruz Biotechnology, USA) for 1 hour at room temperature. Protein bands were detected and quantified using a chemiluminescence kit from pierce biotechnology. GAPDH was detected as the loading control.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software, version 17.0. All values were presented as mean±SD. RL and Cdyn of mice as well as bronchoalveolar lavage cell counts were compared by Independent-Samples Student's t-test .One-way analysis of variance (ANOVA) test was used for statistical analysis of differences in other investigations between groups. $P<0.05$ was considered statistically significant.

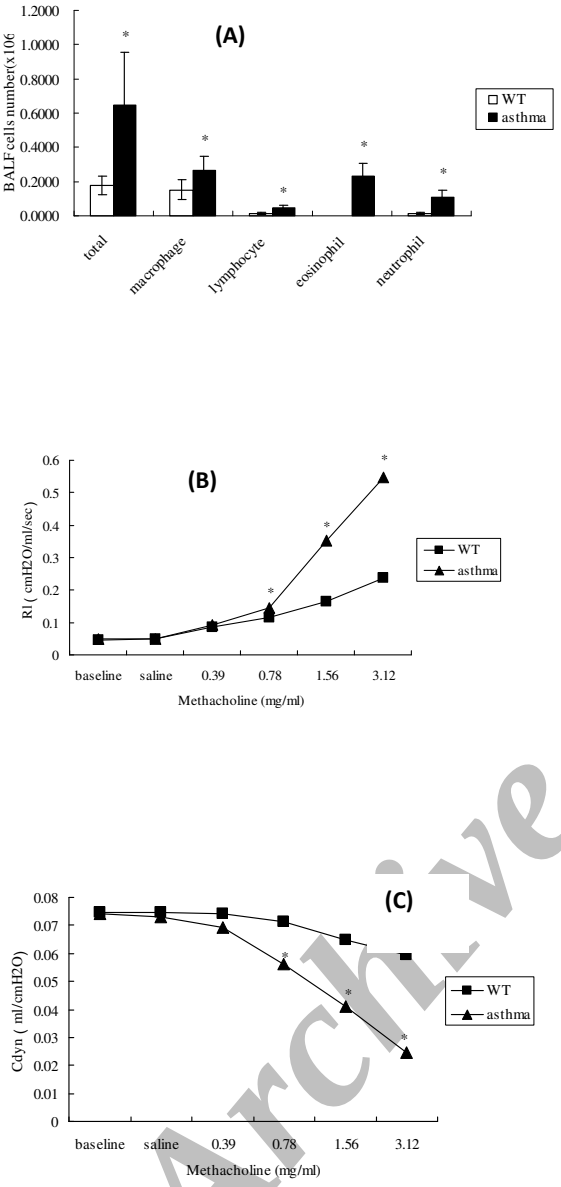

Figure 1. Cell counts in the bronchoalveolar lavage fluid (BALF) and Measurement of airway responsiveness. (A) Cell counts in the BALF in wild-type (WT) mice and asthma mice. (B) Airway resistance (Rl) in WT mice and asthma mice. (C) dynamic lung compliance (Cdyn) in WT mice and asthma mice. Each group included four to five mice. Data represent means ± SD from three independent experiments. *, $p<0.05$ compared to WT mice.

RESULTS

Asthma Model

Twenty-four hours after the last challenge, BAL was performed and airway responsiveness in wild-type (WT) mice and OVA-challenged mice were measured. We found that OVA-challenge induced macrophage, eosinophil, lymphocyte and neutrophil infiltration into the BAL and total cell numbers obviously increased in OVA-challenged mice(Figure1A). As the concentration of methacholine increased, airway resistance of OVA-challenged mice significantly increased compared to WT mice (Figure 1B) and dynamic lung compliance of OVA-challenged mice was poorer than WT mice (Figure 1C),which established that OVA-challenged mice in this study were expected asthma model.

The Expression of β-arrestin2、Phosphorylated-ERK1/2(p-ERK1/2) and IL-17 in $CD4^+$ T Lymphocytes from a Murine Asthma Model

To test β-arrestin2 and p-ERK1/2 expression in $CD4^+$ T lymphocytes from a murine asthma model, Western blot was performed. We found that the expression of β-arrestin2 protein (Figure 2A) and p-ERK1/2 protein (Figure 2B) were greatly increased in $CD4^+$ T lymphocytes from a murine asthma model compared with those from WT mice. Furthermore the production and expression of IL-17 was tested. It was clear that the accumulation of IL-17 in supernatants from cultures of $CD4^+$ T lymphocytes from a murine asthma model was increased compared with those from WT mice(Figure 2C) and the expression of IL-17 mRNA in $CD4^+$ T lymphocytes from a murine asthma model also was increased(Figure 2D). In addition, the expression of IL-17 protein in $CD4^+$ T lymphocytes from a murine asthma model was increased in comparison with those from WT mice (Figure 2E).

The Effect of β-arrestin2 on IL-17 Production and Expression in $CD4^+$ T Lymphocytes from a Murine Asthma Model

To determine the contribution of β-arrestin2 to IL-17 production and expression in $CD4^+$ T lymphocytes from a murine asthma model, $CD4^+$ T lymphocytes from a murine asthma model were transfected with RNA interference specifically targeted to β-arrestin2 (Figure 3A).

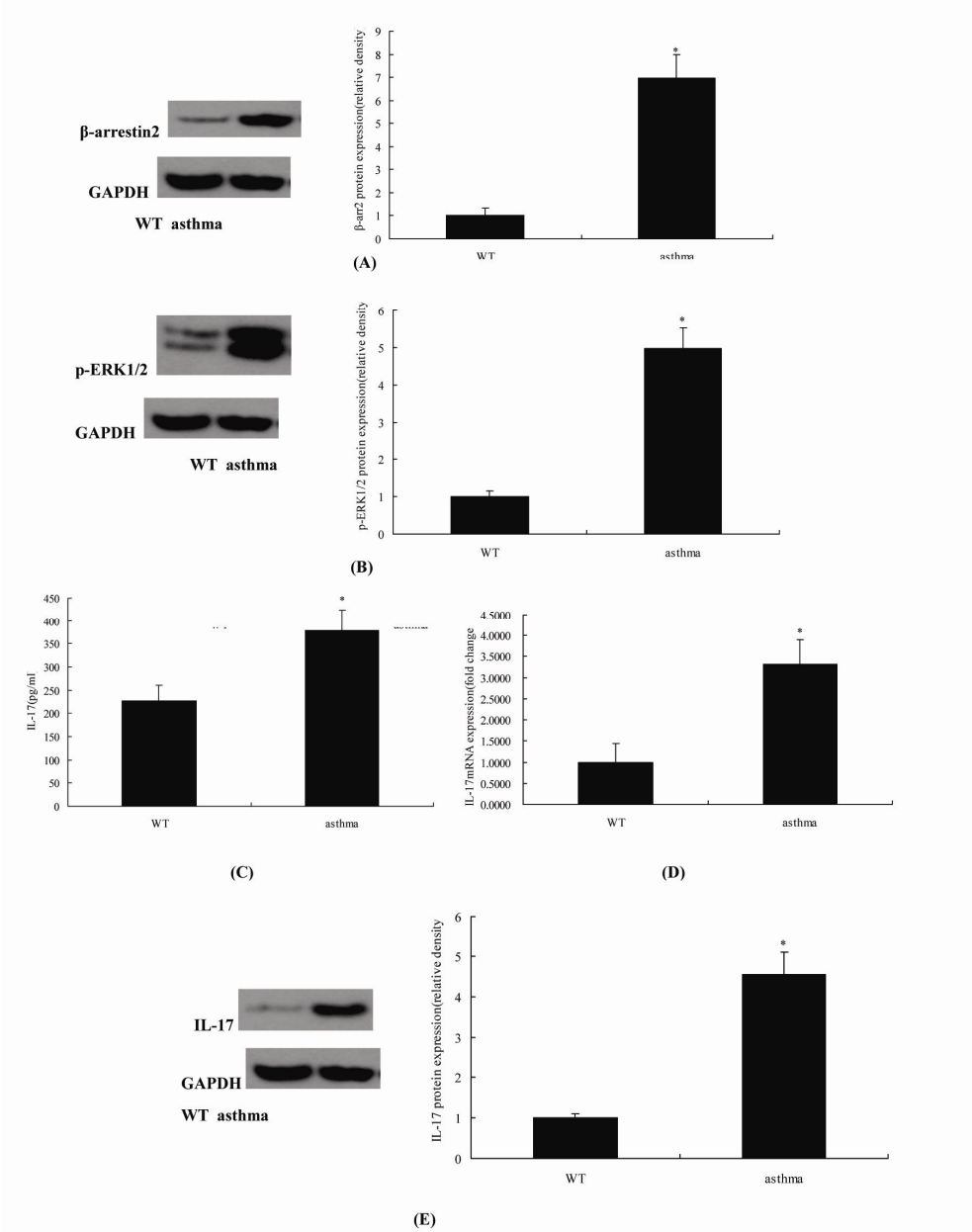

Figure 2. The expression of β-arrestin2(β-arr2)、p-ERK 1/2 and IL-17 in CD4+ T lymphocytes from murine asthmatic models. CD4+ T lymphocytes isolated from WT mice and those from asthmatic models were stimulated with PMA(100ng/ml) and conA(5μg/ml) for 24 h(1×10^6 cells/ml). (A)β-arrestin2 protein expression was analyzed by Western blot analysis. Densitometric analyses are presented as the relative ratio of β-arrestin2 to GAPDH. The relative ratio of β-arrestin2 to GAPDH in WT mice group is arbitrarily presented as 1. (B) P-ERK 1/2 protein expression was analyzed by Western blot analysis. Densitometric analyses are presented as the relative ratio of p-ERK 1/2 to GAPDH. The relative ratio of p-ERK 1/2 to GAPDH in WT mice group is arbitrarily presented as 1. (C)Secretion of IL-17 was determined by ELISA from supernatants of CD4+ T lymphocytes. (D)The expression of IL-17 mRNA was analyzed by real-time PCR. The relative ratio of IL-17 mRNA to β-actin mRNA in WT mice group is arbitrarily presented as 1. (E) IL-17 protein expression was analyzed by Western blot analysis. Densitometric analyses are presented as the relative ratio of IL-17 to GAPDH. The relative ratio of IL-17 to GAPDH in WT mice group is arbitrarily presented as 1.Data represent means ± SD from three independent experiments(five mice per group). *, $p<0.05$ compared to WT mice.

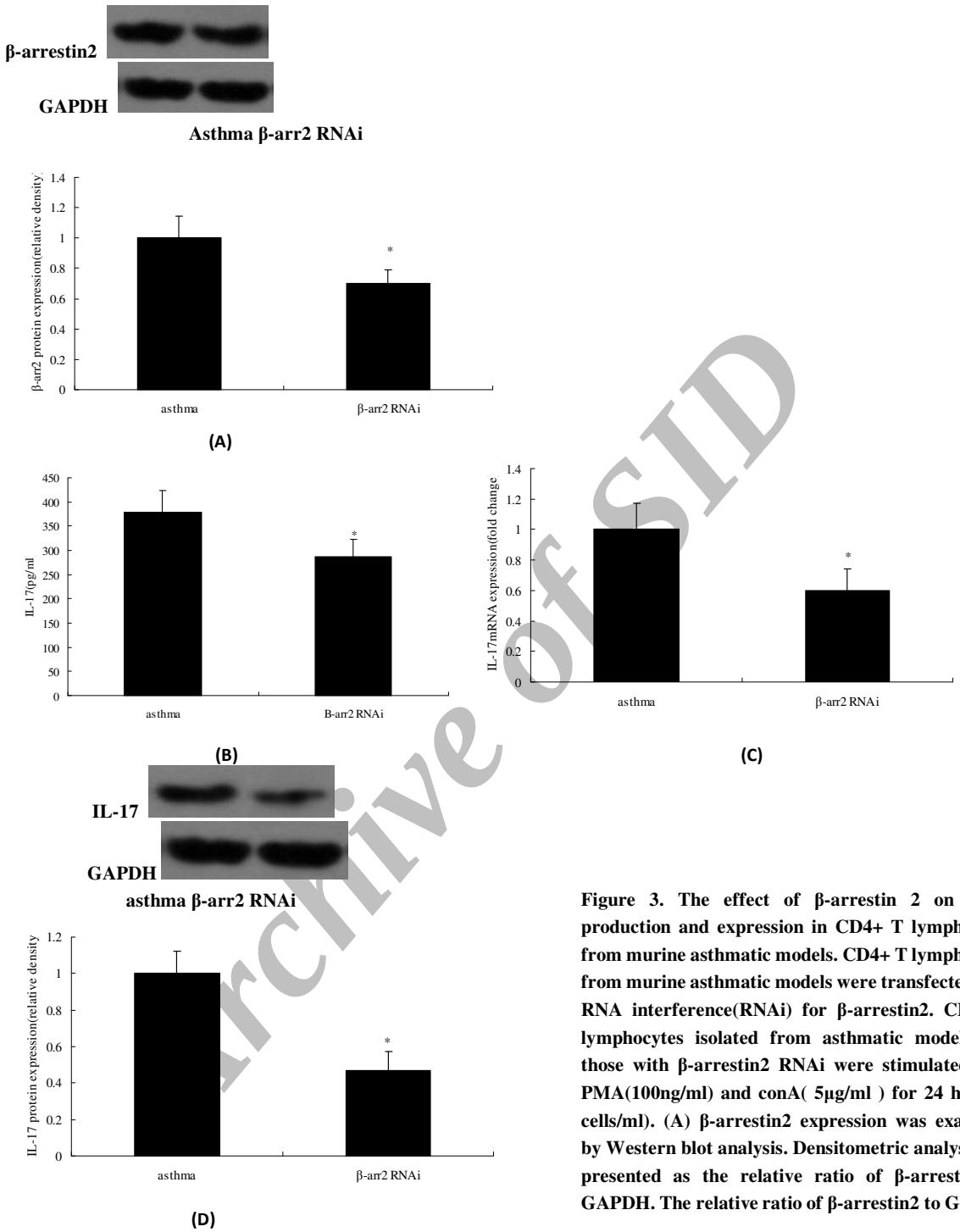

Figure 3. The effect of β-arrestin 2 on IL-17 production and expression in CD4+ T lymphocytes from murine asthmatic models. CD4+ T lymphocytes from murine asthmatic models were transfected with RNA interference(RNAi) for β-arrestin2. CD4+ T lymphocytes isolated from asthmatic models and those with β-arrestin2 RNAi were stimulated with PMA(100ng/ml) and conA(5μg/ml) for 24 h(1×10^6 cells/ml). (A) β-arrestin2 expression was examined by Western blot analysis. Densitometric analyses are presented as the relative ratio of β-arrestin2 to GAPDH. The relative ratio of β-arrestin2 to GAPDH in asthma group is arbitrarily presented as 1. (B) Secretion of IL-17 was determined by ELISA from supernatants of CD4+ T lymphocytes. (C) The expression of IL-17 mRNA was analyzed by real-time PCR. The relative ratio of IL-17 mRNA to β-actin mRNA in asthma group is arbitrarily presented as 1. (D) IL-17 protein expression was analyzed by Western blot analysis. Densitometric analyses are presented as the relative ratio of IL-17 to GAPDH. The relative ratio of IL-17 to GAPDH in asthma group is arbitrarily presented as 1.Data represent means ± SD from three independent experiments(five mice per group). *, $p<0.05$ compared to CD4+ T lymphocytes isolated from asthmatic models.

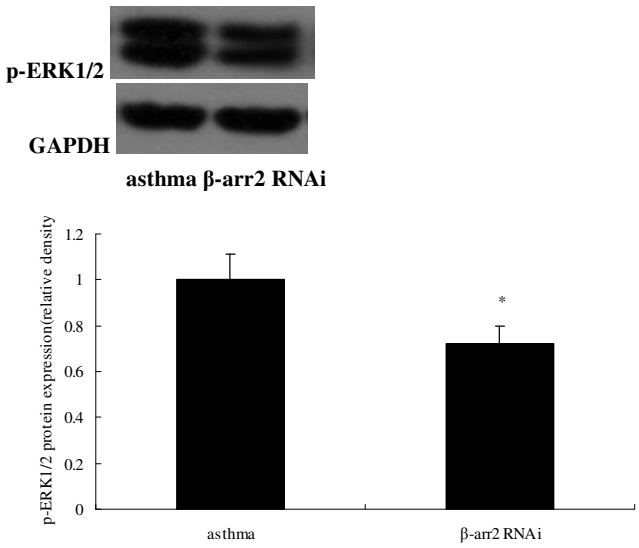

Figure 4. The role of β-arrestin2 in ERK 1/2 activation in CD4+ T lymphocytes from murine asthmatic models. CD4+ T lymphocytes from murine asthmatic models were transfected with RNA interference for β-arrestin2. CD4+ T lymphocytes isolated from asthmatic models and those with β-arrestin2 RNAi were stimulated with PMA(100ng/ml) and conA(5μg/ml) for 24 h(1×10^6 cells/ml). P-ERK 1/2 protein expression was analyzed by Western blot analysis. Densitometric analyses are presented as the relative ratio of p-ERK 1/2 to GAPDH. The relative ratio of p-ERK 1/2 to GAPDH in asthma group is arbitrarily presented as 1. Data represent means ± SD from three independent experiments(five mice per group). *, $p<0.05$ compared to CD4+ T lymphocytes isolated from asthmatic models.

The accumulation of IL-17 in supernatants from cultures of either $CD4^+$ T lymphocytes from a murine asthma model or $CD4^+$ T lymphocytes from a murine asthma model treated with RNA interference specifically targeted to β-arrestin2 was detected.

We found that β-arrestin2 siRNA transfection resulted in a decrease in accumulation of IL-17 in supernatants (Figure 3B). Accordingly, the expression of IL-17 mRNA in $CD4^+$ T lymphocytes from a murine asthma model treated with RNA interference specifically targeted to β-arrestin2 was lower in comparison with those from a murine asthma model (Figure 3C). In addition, western blot confirmed IL-17 protein expression. The expression of IL-17 protein in $CD4^+$ T lymphocytes from a murine asthma model treated with RNA interference specifically targeted to β-arrestin2 was lower in comparison with those from a murine asthma model (Figure 3D).

The Role of β-arrestin2 in ERK 1/2 Activation in $CD4^+$ T Lymphocytes from a Murine Asthma Model

To investigate the role of β-arrestin2 in ERK 1/2 activation in $CD4^+$ T lymphocytes from a murine asthma model, $CD4^+$ T lymphocytes were transfected with RNA interference specifically targeted to β-arrestin2 (Figure 3A).The detection of p-ERK 1/2 was performed. We found that β-arrestin2 siRNA transfection resulted in a decrease in ERK 1/2 phosphorylation in CD4+ T lymphocytes from a murine asthma model (Figure 4).

The Role of P-ERK 1/2 in IL-17 Production and Expression in $CD4^+$ T Lymphocytes from a Murine Asthma Model

To further examine the role of p-ERK 1/2 in IL-17 production in $CD4^+$ T Lymphocytes from a murine asthma model, a specific ERK1/2 inhibitor PD98059 was employed. Pretreatment of $CD4^+$ T lymphocytes from a murine asthma model with PD98059 decreased the accumulation of IL-17 in supernatants (Figure 5A). Accordingly, the expression of IL-17 mRNA in $CD4^+$ T lymphocytes from a murine asthma model pretreated with PD98059 was decreased (Figure 5B) and the expression of IL-17 protein in $CD4^+$ T lymphocytes from a murine asthma model pretreated with PD98059 also was decreased(Figure 5C) .

DISCUSSION

In the present study we showed that the expression of β-arrestin2 and IL-17 in $CD4^+$ T lymphocytes from a murine asthma model was increased compared to those from wild-type (WT) mice, suggesting that the β-arrestin2 and IL-17 were involved in the pathogenesis of the allergic asthma. siRNA depletion of β-arrestin2 decreases ERK 1/2 activation and IL-17 production and expression in $CD4^+$ T lymphocytes from a murine asthma model, suggesting that β-arrestin2 mediated ERK 1/2 activation and stimulated IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model. Moreover, a specific ERK 1/2 inhibitor PD98059 attenuates IL-17 production and expression in $CD4^+$ T lymphocytes from a murine asthma model suggesting that IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model were mediated, in part, by the ERK 1/2 signaling pathway.

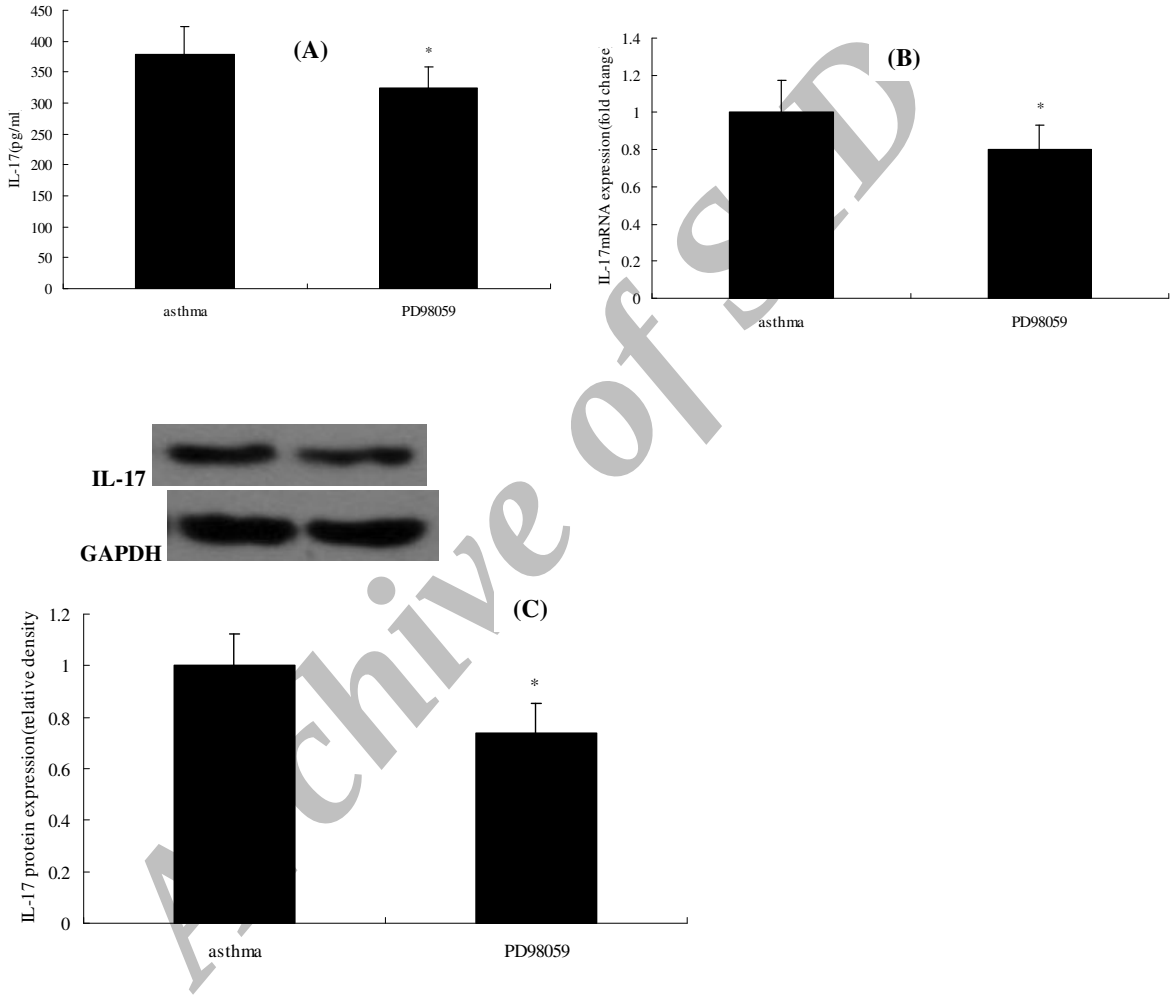

Figure 5. The effect of PD98059 on IL-17 production and expression in CD4+ T lymphocytes from murine asthmatic models. CD4+T lymphocytes from murine asthmatic models were pretreated with or without PD98059(5μM) for 2 h followed by stimulated with PMA(100ng/ml) and conA(5μg/ml) for 24 h(1×10^6 cells/ml). (A) Secretion of IL-17 was determined by ELISA from supernatants of CD4+ T lymphocytes. (B) The expression of IL-17 mRNA was analyzed by real-time PCR. The relative ratio of IL-17 mRNA to β-actin mRNA in asthma group is arbitrarily presented as 1. (C) IL-17 protein expression was analyzed by Western blot analysis. Densitometric analyses are presented as the relative ratio of IL-17 to GAPDH. The relative ratio of IL-17 to GAPDH in asthma group is arbitrarily presented as 1. Data represent means ± SD from three independent experiments(five mice per group). *, $p<0.05$ compared to CD4+ T lymphocytes isolated from asthmatic models.

So we have demonstrated that β-arrestin2 stimulated IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model and the stimulatory effect of β-arrestin2 on IL-17 production and expression was, at least partly, dependent on the ERK 1/2 activation.

β-arrestin2 is ubiquitously expressed in most tissues and plays an important role in regulating signal transduction.[15, 26] The β-arrestin2 -mediated regulation of signal transduction appears to play important roles in cell growth, apoptosis and modulation of immune functions. Julia K.L Walker et al[19] reported allergen-sensitized mice having a targeted deletion of the β-arrestin2 gene neither accumulated T lymphocytes in their airways nor showed other inflammatory features characteristic of asthma, suggesting that β-arrestin2 was essential to the development of allergic asthma and that it exerted its regulatory effect at a proximal step in the inflammatory cascade.[19] Here we showed that the expression of β-arrestin2 was increased in $CD4^+$ T lymphocytes from a murine asthma model compared with those from WT mice, suggesting the involvement of the β-arrestin2 in the pathogenesis of the allergic asthma. The result is consistent with the Julia K.L Walker and co-workers' report.[19]

IL-17 is a pro-inflammatory cytokine produced by recently described Th17 cells, which have been involved in the pathogenesis of the allergic asthma. In our study the production and expression of IL-17 in $CD4^+$ T lymphocytes from a murine asthma model was increased in comparison with those from WT mice. These results agree with previous studies that IL-17 had critical role in the allergic asthma.[10-12] According to our results, the production and expression of IL-17 in $CD4^+$ T lymphocytes from a murine asthma model treated with RNA interference specifically targeted to β-arrestin2 was lower in comparison with those from a murine asthma model, suggesting that β-arrestin2 stimulated IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model. This is the first report that β-arrestin2 stimulate IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model.

Our finding that β-arrestin2 siRNA transfection resulted in a decrease in ERK 1/2 phosphorylation in $CD4^+$ T lymphocytes from a murine asthma model suggested that β-arrestin2 mediated ERK1/2 activation of $CD4^+$ T lymphocytes in a murine asthma model. The result is consistent with previous studies that β-arrestin2 functioned as multifunctional scaffold/adaptor proteins, recruiting Raf-1, MEK, and ERK 1/2 to activate receptors and leading to increased ERK 1/2 activation.[27-29] Furthermore, Our study also showed that PD98059, a specific ERK 1/2 inhibitor, decreased the production and expression of IL-17 in $CD4^+$ T lymphocytes from a murine asthma model suggesting that IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model were mediated by ERK 1/2 signaling pathways. The finding agrees with previous studies that IL-17 expression in T lymphocytes involved several transcription factors as well as activation of MAPK(ERK 1/2, p38 and JNK).[30-32] Thus , in the present study we also demonstrated that the stimulatory effect of β-arrestin2 on IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model is, at least partly, dependent on the ERK 1/2 activation.

Julia K.L Walker et al. study showed that allergen-sensitized mice having a targeted deletion of the β-arrestin2 gene neither accumulated T lymphocytes in their airways nor showed other inflammatory features characteristic of asthma partly because of defective $CD4^+$ T cell migration to the lung.[19] In the present study we demonstrated that β-arrestin2 stimulated IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model, and the stimulatory effect of β-arrestin2 on IL-17 production and expression of $CD4^+$ T lymphocytes in a murine asthma model was, at least partly, dependent on the ERK 1/2 activation. We think the mechanism of β-arrestin2 regulating the development of allergic asthma requires further investigation. It seems plausible that β-arrestin2 could be a valid therapeutic target for the treatment of allergic asthma.

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