

BIOMARKERS

IL-33 promotes airway remodeling and is a marker of asthma disease severityZhi Guo, MD^{1,3}, Jinxiang Wu, MD¹, Jiping Zhao, MD¹, Fen Liu, MD¹, Yingjian Chen, MD², Liquan Bi, MD², Shuying Liu, MD³ and Liang Dong, MD¹¹Department of Pulmonary Medicine, Qilu Hospital of Shandong University, Jinan, China, ²Department of Clinical Laboratory, Jinan Military General Hospital, Jinan, China, and ³Department of Pulmonary Medicine, Jinan Military General Hospital, Jinan, China**Abstract**

Objective: To investigate the function of interleukin-33 (IL-33) in the asthmatic airway remodeling and the relationship between IL-33 and asthma severity. **Methods:** IL-33 levels, sputum eosinophils percentage (EOS%), pulmonary function and total immunoglobulin (IgE) were measured for 45 patients with asthma and 40 non-allergic controls. Asthma severity was assessed. The expressions of IL-33 and reticular basement membrane (RBM) on bronchial biopsy specimens from eight asthma patients and eight non-allergic controls were observed after hematoxylin-eosin staining (HE) and immunohistochemical staining. **In vitro** experiments, real-time polymerase chain reactions and western blotting analysis were used to identify the specific effects of IL-33 administration. **Results:** Serum IL-33 levels in patients with asthma were higher than those in non-allergic controls. Moreover, in asthmatic patients, serum IL-33 levels were negatively correlated to forced expiratory volume in one second (FEV1, % predicted), and positively correlated to asthma severity. Increased expression of IL-33 and RBM thickening were observed on bronchial biopsy specimens obtained from patients with asthma. Serum IL-33 levels were positively correlated to basement membrane thickness. The production of fibronectin1 and type I collagen in human lung fibroblasts (HLF-1) increased at 24 h after IL-33 treatment *in vitro*. Pre-treatment with anti-ST2 antibody or fluticasone propionate (FP) suppressed the production of fibronectin1 and types I collagen induced by IL-33. **Conclusions:** IL-33 is a marker of asthma severity, and may contribute to airway remodeling in asthma by acting on human lung fibroblasts.

Keywords

Airway remodeling, asthma, HLF-1, IL-33, ST2

History

Received 10 January 2014

Revised 29 April 2014

Accepted 30 April 2014

Published online 5 June 2014

Introduction

Asthma is a chronic inflammatory disorder of the airway wall. Many cells such as eosinophils, mast cells, Th2 cells and epithelial cells play important roles through the release of various mediators during an asthma attack. Interleukin-33 (IL-33) was originally identified as a “nuclear factor from high endothelial venules (NF-HEV)” expressed in endothelial cell nuclei [1]. Through IL-33 receptor ST2, IL-33 activates NF-kappaB and MAP kinases, and drives the production of pro-inflammatory cytokines IL-5 and IL-13 released from polarized Th2 cells [2]. Lee et al. [3] reported that anti-IL-33 antibody and sST2 suppressed the IL-33-mediated allergic airway inflammation in a murine asthma model. These data highlighted that IL-33 played a crucial rule in airway inflammation. Airway remodeling is an important

pathological feature of asthma, including subepithelial fibrosis, matrix protein deposition, goblet cell hyperplasia, glandular hypertrophy, hypertrophy of smooth muscle cells and epithelial injury [4–6]. Previous studies have shown that human lung fibroblast might contribute to reticular basement membrane (RBM) thickening in asthma. Human lung fibroblast plays an important role in the development of RBM thickening by altering its phenotype from a quiescent cell into myofibroblast, and myofibroblast can secrete proinflammatory mediators and extracellular matrix proteins, including collagens I, III, and V, fibronectin, and so on [7–10].

Many studies showed that the IL-33/ST2 pathway correlated with asthma severity [11,12], but the mechanism of the association between IL-33 and asthma severity remains unclear. We hypothesized that IL-33 could induce airway remodeling by acting on human lung fibroblasts. Firstly, we investigated the expression of IL-33 in patients with asthma and the relationship between IL-33 and asthma severity. Then we measured the mRNA and protein expression of fibronectin1 and type I collagen from human lung fibroblast cell, normal (HLF-1) *in vitro* after IL-33 treatment. These findings suggested that IL-33 might promote airway remodeling in asthma and was a marker of asthma severity.

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Table 1. Characteristics of patients.

	Asthma group (n = 45)	Control group (n = 40)
Male/Female	24/21	23/17
Age (year)	39.56 ± 5.27	43.55 ± 8.86
Sputum EOS (%)	21(4-50) (n = 23)	NA
Total IgE (IU/ml)	86.95 (17.8–709)*	31.50 (14–156)
FVC (% predicted)	96.08 ± 18.29	102.37 ± 7.49
FEV1 (% predicted)	64.32 ± 18.33**	86.12 ± 11.47
FEV1/FVC (%)	58.68 ± 17.33**	84.35 ± 12.61

The patients in asthma group were defined by Global Initiative for Asthma 2006, and allergic diseases or any inflammatory diseases were excluded in the control group.

p* < 0.05 versus Controls; *p* < 0.01 versus controls.

Methods

Subjects

We recruited 45 patients with asthma (asthma group) from the Outpatient Clinic of Jinan Military General Hospital, as defined by Global Initiative for Asthma 2006. None of the patients had used corticosteroids within 4 weeks before entry. The other group involved 40 non-allergic controls (control group) with no signs of allergic diseases or any inflammatory diseases. Because there was no or less sputum, only 23 patients in the asthma group accomplished sputum eosinophil count tests. Sputum induction was carried out as previously described [13]. Sputum samples were collected and Wright–Giemsa staining was performed within two hours. Results were expressed as the percentage of eosinophils in the total nucleated cells count (Table 1). Pulmonary function tests were performed with a spirometer (Jaeger, Bavaria, Germany) for forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and FEV1/FVC (Table 1). Asthma severity was assessed according to Global Initiative for Asthma 2006. In this study, based on the clinical manifestations and lung function, asthma severity was classified into mild severity (Intermittent and Mild Persistent), moderate severity (Moderate Persistent) and severe severity (Severe Persistent).

Reagents

Recombinant human IL-33 was purchased from PeproTech EC Ltd. (London, UK). A monoclonal mouse anti-human ST2/IL-1 R4 neutralizing antibody (IgG1, Clone 97203) was purchased from R&D Systems, Inc. (Minneapolis, MN). Fluticasone propionate (FP) was purchased from Melone Pharmaceutical Co Ltd. (Dalian, China).

Blood samples and human tissue

This study was approved by the ethics committee of Jinan Military General Hospital, and informed consent was obtained from all participating subjects. Venous blood samples were obtained from 45 patients in the asthma group and 40 individuals in the control group. Venous blood samples were centrifuged at 4000g at room temperature for 15 min. Then, the separated serum samples were preserved at –20 °C till measurements. Bronchial mucosal tissues were obtained from eight patients in the asthma group and

eight subjects in the control group. Biopsy procedure and processing were carried out as previously described [14]. Several bronchial mucosa biopsy specimens were obtained using alligator forceps at the opening of the right upper and middle lobe. Each specimen was fixed in 10% buffered formalin solution at room temperature and embedded in paraffin.

Total IgE and IL-33 levels

Total serum immunoglobulin (IgE) and IL-33 levels of the two groups were measured with Human IL-33 Quantikine ELISA Kit (R&D Systems, sensitivity: 1.65 pg/ml, assay range: 6.25–400 pg/ml) and N Latex IgE mono (Siemens, Munich, Germany) according to the manufacturer’s protocol.

Hematoxylin-eosin staining and immunocytochemistry

Paraffin-embedded specimens were sectioned at 5-μm thickness with a microtome, and stained with hematoxylin-eosin (ZSGB-BIO, Beijing, China). Some specimens were stained immunohistochemically for IL-33. They were incubated overnight at 4 °C with a polyclonal rabbit anti-human IL-33 antibody (2 μg/ml, MBL, Nagoya, Japan). After being washed, sections were incubated with a horseradish peroxidase-conjugated secondary antibody (ZSGB-BIO, Beijing, China) for 60 min at room temperature. The nuclei were counterstained with hematoxylin. To confirm specificity of the IL-33 antibody, normal rabbit serum (ZSGB-BIO, Beijing, China) was used as negative antibody controls.

Cell culture and treatment

HLF-1 (a gift from Qilu Hospital of Shandong University) was cultured in Dulbecco’s modified Eagle’s medium (DMEM, Hyclone, Logan, UT) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY), 100 U/ml penicillin and 100 μg/ml streptomycin. They were then incubated with 5% CO₂ at 37 °C. Cells at the third passage were used for the experiment. Cells were seeded at 1 × 10⁵ cells/well in 6-well culture plates and cultured till 50–60% confluence. The cells were starved in DMEM containing 5% FBS overnight, and then stimulated with IL-33 (1 ng/ml, 10 ng/ml or 100 ng/ml) for 24 h. In some experiments, cells were pretreated for 1 h with a monoclonal mouse anti-human ST2/IL-1 R4 neutralizing antibody (10 μg/ml, R&D, Minneapolis, MN) or FP (100 nm, Melone, China) before stimulation with 100 ng/ml IL-33.

Quantitative real-time PCR

Total RNA was isolated from HLF-1 using the RNAiso Plus (Takara, Shiga, Japan). cDNA was synthesized using the PrimeScript™ RT reagent Kit (Takara, Shiga, Japan). Quantification of mRNA levels was performed by real-time PCR using Roche LightCycler480 and SYBR® Premix Ex Taq™ II (Takara, Shiga, Japan). Amplification conditions were 40 cycles of 30 s at 95 °C, 5 s at 95 °C, 20 s at 60 °C. β-Actin was used as an internal control. Primers were designed to span an intron and the sequences were as follows (5’ to 3’): TGCGTGACATTAAGGAGAAG (β-actin sense),

GTCAGGCAGCTCGTAGCTCT (β -actin antisense), GAGC TATTCCCTGCACCTGATG (fibronectin1 sense), CGTGCA AGGCAACCACACT (fibronectin1 antisense), GCCTAGCAACATGCCAATC (types I collagen sense) GCAAAGTTCCCACCGAGA (types I collagen antisense), AGGCTTTTCTCTGTTTCCAGTAATCGG (ST2 sense) and CAGTGACACAGAGGGAGTTCATAAAGTTAGA (ST2 antisense).

Western blot analysis

Cells were rinsed in ice-cold PBS and incubated in RIPA buffer (Kangchen, Shanghai, China) containing the mini complete protease inhibitor cocktails (Kangchen, Shanghai, China) for 15 min on ice. Extracts were clarified at $14\,000 \times g$ at 4°C for 15 min and protein concentration was determined using the Bradford assay (Solarbio, Beijing, China). Thirty micrograms of total proteins were resolved on 12% SDS-PAGE gels (Beyotime, Shanghai, China) and transferred onto nitrocellulose membranes (Millipore Co., Milford, MA). The membranes were blocked using 10% skim milk in PBS. Blots were incubated with a polyclonal rabbit anti-human fibronectin1 (FN1) antibody ($2\,\mu\text{g}/\text{ml}$, Proteintech, Chicago, IL), a polyclonal rabbit anti-human types I collagen (COL-I) antibody ($2\,\mu\text{g}/\text{ml}$, Santa Cruz, CA), a polyclonal rabbit anti-human β -actin antibody ($0.5\,\mu\text{g}/\text{ml}$, Bioworld, Louis Park, MN), or a monoclonal mouse anti-human ST2/IL-1 R4 neutralizing antibody ($1\,\mu\text{g}/\text{ml}$, R&D, Minneapolis, MN) overnight at 4°C . Membranes were washed and treated with an HRP-conjugated polyclonal goat anti-rabbit antibody (ZSGB-BIO, Beijing, China) or a polyclonal goat anti-mouse

antibody (ZSGB-BIO, Beijing, China) for 1 h at room temperature. Signal was developed using the ECL Plus kit (Millipore, Bedford, MA). Densitometric analysis of protein signal was performed using Quantity One 4.6.2 (BIO-RAD, Hercules, CA).

Data analyses

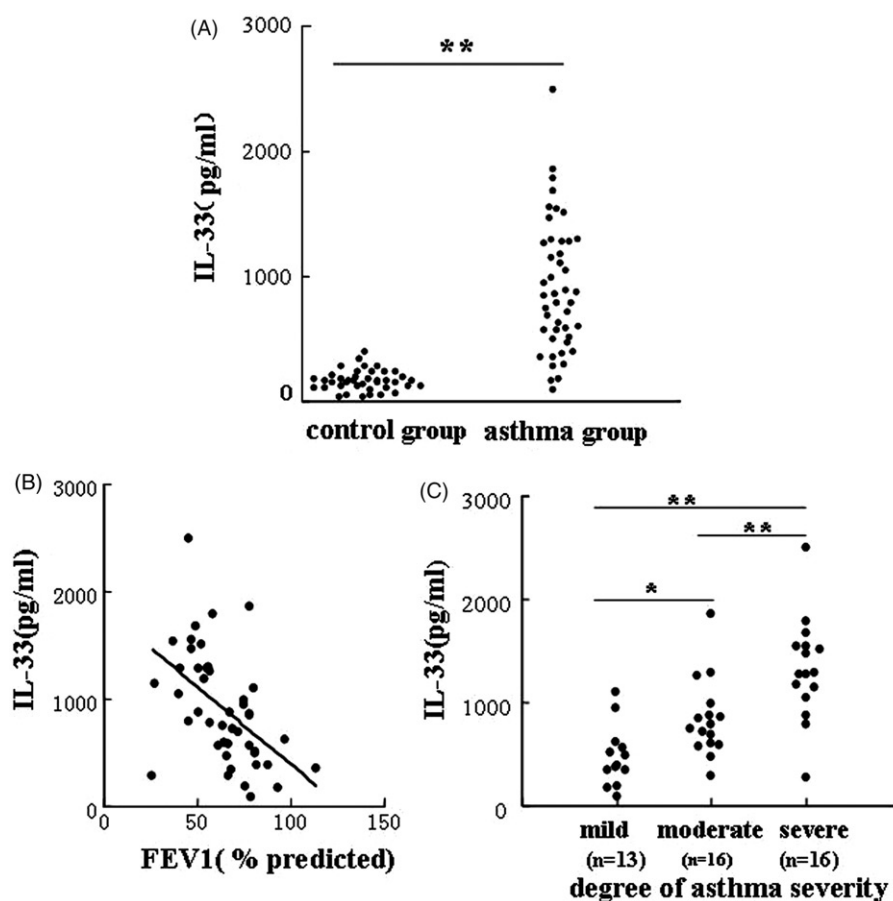
All data are expressed as mean $\pm$ SE. Differences between groups were tested by the ANOVA and an unpaired two-tailed Student's *t*-test (SPSS 17.0, SPSS Inc., Chicago, IL). Differences resulting in *p* values of less than 0.05 were considered statistically significant.

Results

Expression of serum IL-33 in asthma patients and its correlation with FEV1% and asthma severity

To evaluate serum levels of IL-33 in asthma patients, we performed ELISA using sera of 45 patients in the asthma group and 40 patients in the control group. The mean serum IL-33 level of patients with asthma and controls was $903.62 \pm 523.78\,\text{pg}/\text{ml}$ and $158.1 \pm 81.74\,\text{pg}/\text{ml}$, respectively. Patients with asthma had significantly higher levels of serum IL-33 than those of controls (Figure 1A, $p < 0.01$). Total IgE was higher in the asthma group than in the control group (Table 1, $p < 0.05$), and FEV1 (% predicted) was significantly lower in the asthma group than in the control group (Table 1, $p < 0.01$). Serum levels of IL-33 was significantly correlated with FEV1 (% predicted) (Figure 1B, $r_s = -0.571$, $p < 0.01$). Furthermore, a significant correlation was found between

Figure 1. Expression of serum IL-33 in asthma patients and its correlation with FEV1% and asthma severity. (A) Serum IL-33 level was significantly increased in patients with bronchial asthma compared to those in the controls ($p < 0.01$). (B) The correlation between serum level of IL-33 and FEV1 (% predicted) ($r_s = -0.571$, $p < 0.01$). (C) The correlation between serum level of IL-33 and asthma severity ($r_s = 0.691$, $p < 0.01$). * $p < 0.05$ versus Controls; ** $p < 0.01$ versus controls.



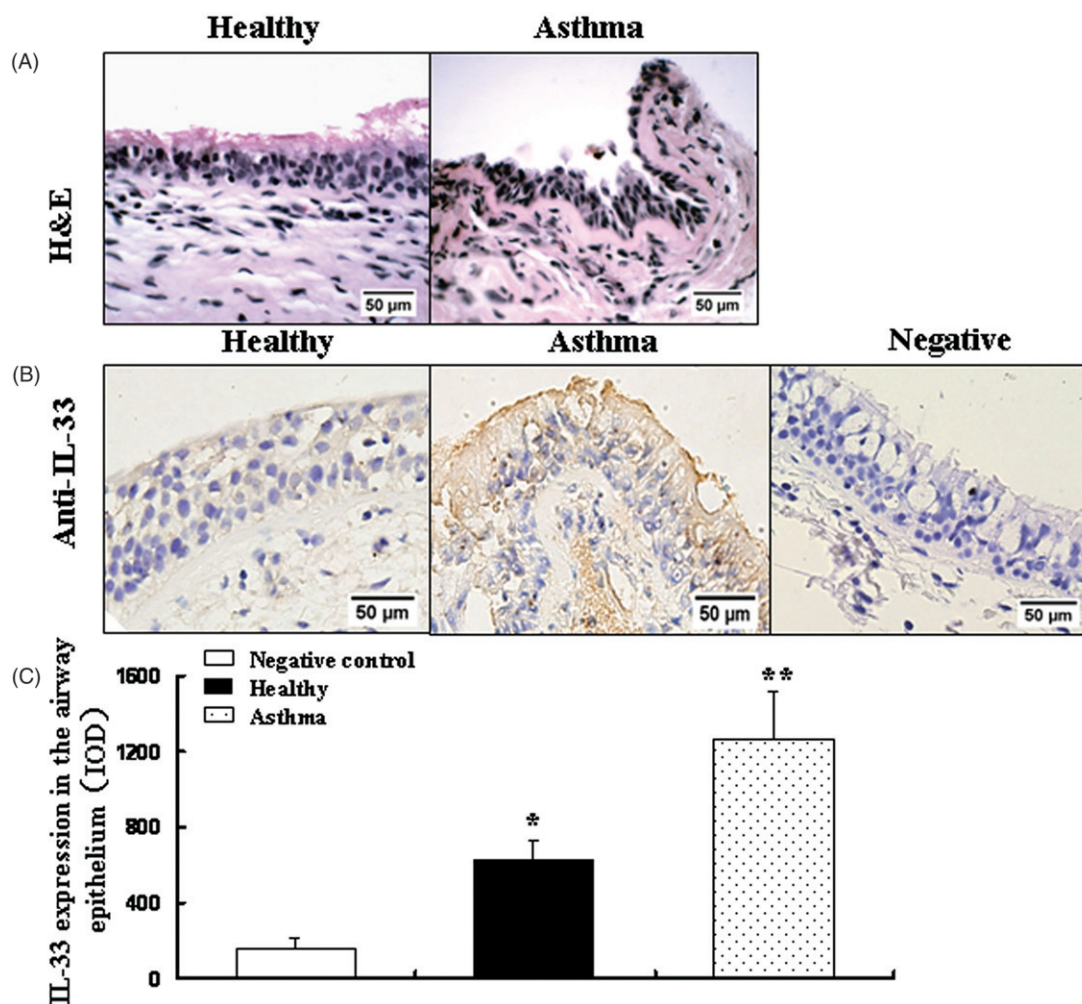


Figure 2. Hematoxylin-eosin and immunohistochemical staining of bronchial mucosal tissues. (A) The thickness of RBM was increased in patients with asthma. (B) Immunoreactivity for IL-33 was observed not only in the nuclei but also in the cytoplasm of epithelia from patients with asthma

serum level of IL-33 and asthma severity in the asthma group (Figure 1C, $rs=0.691$, $p<0.01$). However, the correlation between serum level of IL-33 and total IgE was not significant ($rs=0.177$, $p=0.455$). Serum level of IL-33 failed to correlate with sputum eosinophils percentage (EOS%) ($rs=0.217$, $p=0.576$).

The high expression of IL-33 in asthma patients

HE staining demonstrated that there were subepithelial fibrosis, collagen deposition and RBM thickening in patients with asthma (Figure 2A). IL-33 positive cells were quantified by densitometry using the Image-Pro Plus6.0 (Media Cybernetics, Bethesda, MD). The nuclei and (or) the cytoplasm of epithelia stained in brown indicated IL-33-positive cells and all measurements were performed by a pathologist in a blinded manner. The expression of IL-33 was higher significantly in the epithelium of asthmatic patients compared with controls (Figure 2B and C, $p<0.01$).

Increased IL-33 expression in asthma patients was positively correlated with the thickness of RBM

To investigate the relationship between IL-33 and the thickness of basement membrane, we measured the thickness

of RBM from biopsy specimens stained with HE in the asthma group ($n=8$) and the control group ($n=8$). The thickness of RBM was assessed as following: RBM thickness was calculated as area/length of RBM. The area of RBM (from the base of the bronchial epithelium to the outer limit of the reticular lamina) and the length of RBM were measured by a computerized image. The mean values of 3–5 measurements in two stained sections per subject were used for analysis. All measurements were performed by a pathologist in a blinded manner. The thickness of RBM was higher in the asthma group than in the control group (Figure 3A, $p<0.05$). Furthermore, a correlation was found between the serum level of IL-33 and the thickness of RBM in the asthma group (Figure 3B, $rs=0.831$, $p<0.05$).

IL-33 can stimulate the induction of ST2 in human lung fibroblast

To detective ST2 expression in HLF-1, we observed the expression of ST2 by western blot analysis (Figure 4A). To confirm the expression of ST2 in HLF-1, human bronchial epithelial cell (BEAS-2B, a gift from Qilu Hospital of Shandong University) was used as positive control (Figure 4A) [15]. The presence of ST2 in HLF-1 was

Figure 3. (A) The thickness of RBM ranged from 2.5 to 10.25 μm (median: 7.17 μm) in the asthma group and 1.21 to 6.15 μm (median: 3.29 μm) in the control group. Bold lines represent median. (B) The correlation between serum level of IL-33 and the thickness of RBM of eight patients in the asthma group ($r_s = 0.831$, $p < 0.05$). * $p < 0.05$ versus controls.

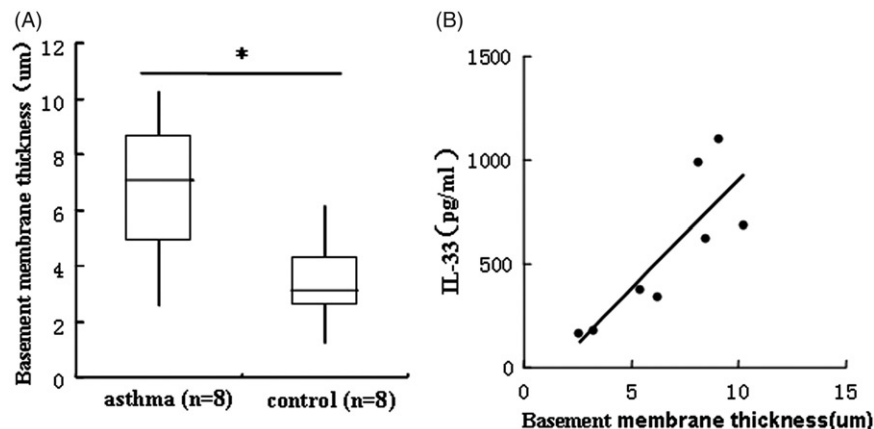


Figure 4. Expression of ST2 in HLF-1 *in vitro*. (A) The constitutive expression of ST2 in HLF-1 (human bronchial epithelial cell as a positive control). (B) The relative mRNA expression of ST2 by HLF-1 exposed to IL-33 in dose response. Error bars represent mean $\pm$ SE; $n = 3$ of different experiments. ** $p < 0.01$ versus controls.

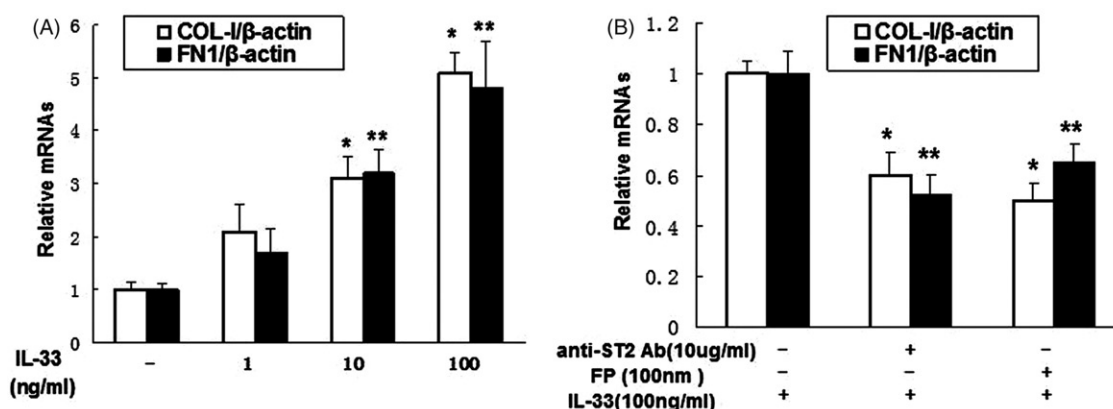
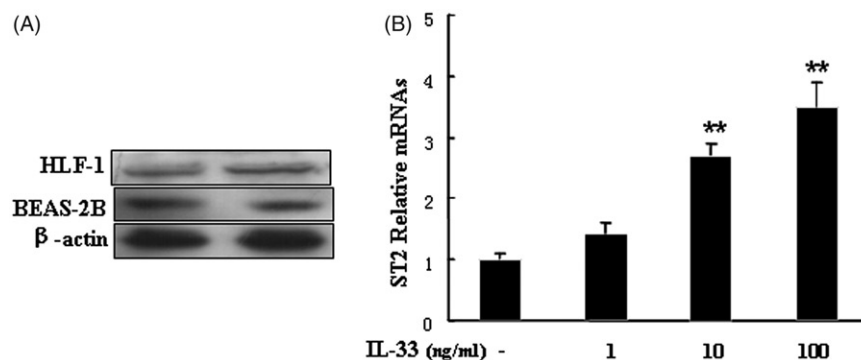


Figure 5. The relative mRNA expression of FN1 and COL-I in cultured HLF-1 *in vitro*. (A) The relative mRNA of FN1 and COL-I were increased 24 h after treatment with 10 or 100 ng/mL IL-33. (B) Pre-treatment with 10 $\mu\text{g}/\text{ml}$ anti-ST2 Ab or 100 nm FP inhibited the IL-33-mediated mRNA expression of FN1 and COL-I. Error bars represent mean $\pm$ SE; $n = 3$ of different experiments. ** $p < 0.01$ versus Controls; * $p < 0.05$ versus controls.

further confirmed by qRT-PCR. As shown in Figure 4(B), the relative mRNA expression of ST2 was increased in dose-response after IL-33 treatment (Figure 4B). The findings indicated that IL-33 could stimulate the induction of ST2 in HLF-1.

IL-33 can promote airway remodeling via ST2 in HLF-1 *in vitro*

To investigate the functional relationship between IL-33 and airway remodeling, we assessed the response of HLF-1 to IL-33 *in vitro*. When cultured HLF-1 were treated with

1–100 ng/mL IL-33 for 24 h, we found the production of fibronectin1 and types I collagen were increased (Figures 5 and 6). Firstly, we demonstrated that the mRNA of fibronectin1 and types I collagen were increased 24 h after treatment with 10 or 100 ng/mL IL-33 by qRT-PCR (Figure 5A). Pre-treatment with 10 $\mu\text{g}/\text{ml}$ anti-ST2 Ab or 100 nm FP can suppress the mRNA expression of fibronectin1 and types I collagen induced by 100 ng/mL IL-33 (Figure 5B). Furthermore, as shown in Figure 6, the fibronectin1 and types I collagen proteins were increased 24 h after 10 or 100 ng/mL IL-33 treatment (Figure 6A and B). Pre-treatment with 10 $\mu\text{g}/\text{ml}$ anti-ST2 Ab or 100 nm FP suppressed the IL-33-

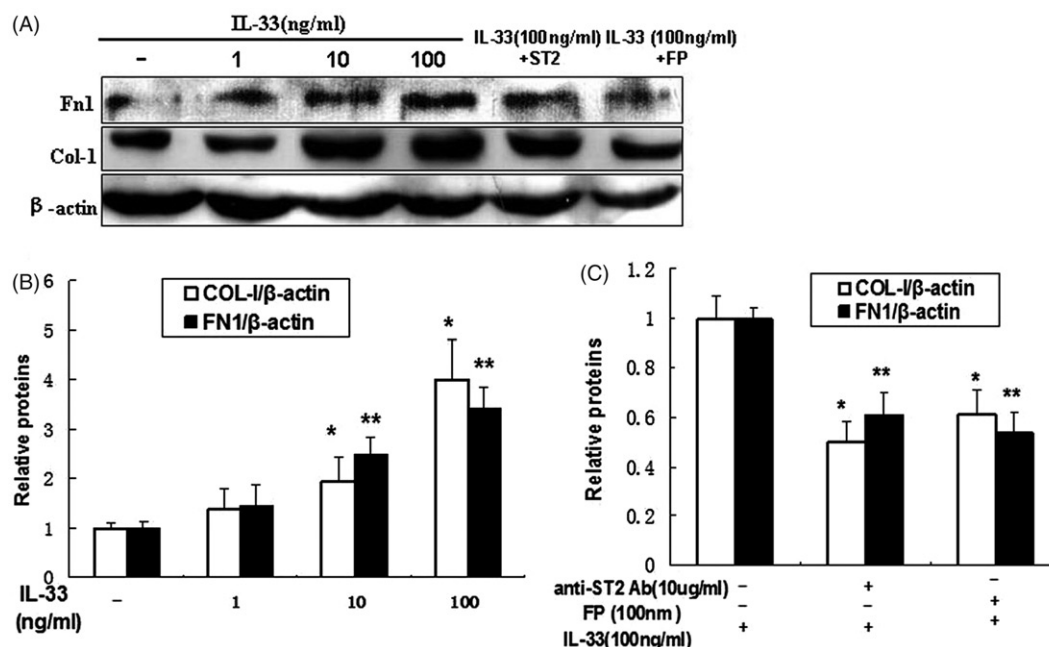


Figure 6. The relative protein expression of FN1 and COL-I in cultured HLF-1 *in vitro*. (A) Western blot analyses for FN1 and COL-I in cultured HLF-1 *in vitro*. (B) The relative proteins of FN1 and COL-I were significantly increased 24 h after IL-33 treatment. (C) Pre-treatment with 10 μg/ml anti-ST2 Ab or 100 nm FP significantly inhibited the IL-33-mediated expression of FN1 and COL-I proteins. Error bars represent mean ± SE; $n = 3$ of different experiments. ** $p < 0.01$ versus Controls; * $p < 0.05$ versus controls.

mediated protein expressions of fibronectin1 and types I collagen (Figure 6C).

Discussion

IL-33 is produced early during the allergic inflammatory process and induces Th2 activation through type 2 innate lymphoid cells, which express IL-13 and IL-4 and promote type-2 immune activity [2]. Serum IL-33 level has previously been demonstrated to be elevated in asthmatic patients [12,16]. Moreover, as reported in other organs, bronchial epithelium is an important IL-33 reservoir in the lung [16]. Such results are in accordance with our findings of increased IL-33 levels in asthmatic patients and increased IL-33-immunoreactivity in the epithelium. However, unlike previous results [17], we showed that IL-33-immunoreactivity was present not only in the nuclei but also in the cytoplasm of epithelium from patients with asthma.

A positive correlation was observed between serum level of IL-33 and asthma severity. This finding is supported by recent reports of the association between IL-33 level in airway smooth muscle cells or induced sputum and disease severity [12,18]. Pulmonary function is one of the most objective factors in the criteria of asthma severity in Global Initiative for Asthma 2006. Our data indicated that the positive correlation between IL-33 and asthma severity was depended on the negative correlation between IL-33 and pulmonary function. There has been evidence from experimental models that IL-33 stimulates differentiation of eosinophils [19–21], function of mast cells [22], expansion of IL-13-producing type 2 innate lymphoid cells [23], airway hyper-responsiveness and bronchoconstriction. However, consistent with another previous study [24], we failed to observe any correlation among sputum EOS%, total IgE and serum

level of IL-33. These findings indicated that IL-33 promoted decreased pulmonary function by other pathways.

Airway remodeling is an important feature of asthma. The thickened bronchial epithelial basement membrane is a histopathologic characteristic of asthma. Our histological findings in this study were in agreement with the results in asthmatic patients in terms of degree of sub-epithelial fibrosis, collagen deposition and RBM thickness [7,25]. The thickness of RBM is resulted from the increased deposition of types I, III, and V collagens and fibronectin [7,26,27], and correlated with FEV1 and disease severity [28–31]. In addition, Saglani et al. [32] found that IL-33 promoted RBM thickening in children with severe therapy-resistant asthma. Therefore, we further hypothesized that IL-33 might promote RBM thickening and airway remodeling in asthma.

To confirm the correlation between IL-33 and RBM thickness, we assessed the response of HLF-1 to IL-33 *in vitro*. Unlike the results obtained in adults [32], we found that IL-33 induced the production of fibronectin1 and types I collagen from HLF-1 *in vitro*. Moreover, our data also showed that ST2 was constitutively stimulated by IL-33 in a dose-dependent manner in HLF-1. In addition, the production of fibronectin1 and types I collagen were markedly blocked by anti-ST2 Ab. These findings suggested that IL-33 acted its function on airway remodeling via ST2 in HLF-1. Interestingly, pre-treatment with FP similarly suppressed the production of fibronectin1 and types I collagen induced by IL-33 treatment. The absence of a-smooth muscle actin release and myofibroblast phenotype after IL-33 stimulation [32] likely explained that the effect of IL-33 in remodeling is reversible. Therefore, early intervention is critical to asthma control.

In summary, we showed that IL-33 was a marker of asthma severity. IL-33 may promote airway remodeling in asthma by

acting on human lung fibroblasts. The IL-33/ST2 signaling pathway might be a therapeutic target that specifically attenuates airway remodeling in asthma.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

This study was supported by National Nature Science Foundation of China (Grant No.: 81070016, 81270072) and the Natural Science Foundation of Shandong Province (Grant No.: ZR2011HM020).

References

- Baekkevold ES, Roussigne M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, Brandtzaeg P, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol* 2003;163:69–79.
- Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, et al. IL-33, an interleukin 1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;23:479–490.
- Lee HY, Rhee CK, Kang JY, Byun JH, Choi JY, Kim SJ, Kim YK, et al. Blockade of IL-33/ST2 ameliorates airway inflammation in a murine model of allergic asthma. *Exp Lung Res* 2014;40:66–76.
- Broide DH. Immunologic and inflammatory mechanisms that drive asthma progression to remodeling. *J Allergy Clin Immunol* 2008;121:560–570.
- Kaminska M, Foley S, Maghni K, Storness-Bliss C, Coxson H, Ghezzi H, Lemièrre C, et al. Airway remodeling in subjects with severe asthma with or without chronic persistent airflow obstruction. *J Allergy Clin Immunol* 2009;124:45–51e1–e4.
- Pascual RM, Peters SP. Airway remodeling contributes to the progressive loss of lung function in asthma: an overview. *J Allergy Clin Immunol* 2005;116:477–486.
- IRoche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;1:520–524.
- Huang J, Olivenstein R, Taha R, Hamid Q, Ludwig M. Enhanced proteoglycan deposition in the airway wall of atopic asthmatics. *Am J Respir Crit Care Med* 1999;160:725–729.
- Wilson JW, Li X. The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. *Clin Exp Allergy* 1997;27:363–371.
- Karjalainen EM, Lindqvist A, Laitinen LA, Kava T, Altraja A, Halme M, Laitinen A. Airway inflammation and basement membrane tenascin in newly diagnosed atopic and nonatopic asthma. *Respir Med* 2003;97:1045–1051.
- Oshikawa K, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, Ohno S, Tominaga SI, Sugiyama Y. Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. *Am J Respir Crit Care Med* 2001;164:277–281.
- Préfontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, Lemièrre C, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *Immunol* 2009;183:5094–5103.
- Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE, Dolovich J. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992;47:25–29.
- Feltis BN, Wignarajah D, Zheng L, Ward C, Reid D, Harding R, Walters EH. Increased vascular endothelial growth factor and receptors: relationship to angiogenesis in asthma. *Am J Respir Crit Care Med* 2006;173:1201–1207.
- Tajima S, Bando M, Ohno S, Sugiyama Y, Oshikawa K, Tominaga S, Itoh K, et al. ST2 gene induced by type 2 helper T cell (Th2) and proinflammatory cytokine stimuli may modulate lung injury and fibrosis. *Exp Lung Res* 2007;33:81–97.
- Préfontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, Martin JG, Hamid Q. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010;125:752–754.
- Moussion C, Ortega N, Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel “alarmin”? *PLoS One* 2008;3:e3331.
- Hamzaoui A, Berraies A, Kaabachi W, Haifa M, Ammar J, Kamel H. Induced sputum levels of IL-33 and soluble ST2 in young asthmatic children. *J Asthma* 2013;50:803–809.
- Kim HY, Chang YJ, Subramanian S, Lee HH, Albacker LA, Matangkasombut P, Savage PB, et al. Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity. *J Allergy Clin Immunol* 2012;129:216–227e1–e6.
- Barlow JL, Bellosi A, Hardman CS, Drynan LF, Wong SH, Cruickshank JP, McKenzie AN. Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. *J Allergy Clin Immunol* 2012;129:191–198e1–e4.
- Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, Liew FY. IL-33 exacerbates eosinophil-mediated airway inflammation. *J Immunol* 2010;185:3472–3480.
- Lai Y, Altemeier WA, Vandree J, Piliponsky AM, Johnson B, Appel CL, Frevert CW, et al. Increased density of intraepithelial mast cells in patients with exercise-induced bronchoconstriction regulated through epithelially derived thymic stromal lymphopoietin and IL-33. *J Allergy Clin Immunol* 2014;133:1448–55.
- Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, Bucks C, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol* 2013;132:933–941.
- Fux M, Pecaric-Petkovic T, Odermatt A, Hausmann OV, Lorentz A, Bischoff SC, Virchow JC, Dahinden CA. IL-33 is a mediator rather than a trigger of the acute allergic response in humans. *Allergy* 2014;69:216–222.
- Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1990;3:507–511.
- Laitinen A, Altraja A, Kampe M, Linden M, Virtanen I, Laitinen LA. Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid. *Am J Respir Crit Care Med* 1997;156:951–958.
- Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma: an ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989;140:1745–1753.
- Chetta A, Fores IA, Del Donno M, Bertorelli G, Pesci A, Olivieri D. Airways remodelling is a distinctive feature of asthma and is related to severity of disease. *Chest* 1997;111:852–857.
- Sont JK, Willems LNA, Bel HE, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guideline to long-term treatment. *Am J Respir Crit Care Med* 1999;159:1043–1051.
- Kasahara K, Shiba K, Ozawa T, Okuda K, Adachi M. Correlation between the bronchial subepithelial layer and whole airway wall thickness in patients with asthma. *Thorax* 2002;57:242–246.
- Payne DN, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, Jeffery PK. Early thickening of the reticular basement membrane in children with difficult asthma. *Am J Respir Crit Care Med* 2003;167:78–82.
- Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, Denney L, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol* 2013;132:676–685e13.