



The effects of Th2 cytokines on the expression of ADAM33 in allergen-induced chronic airway inflammation[☆]

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ABSTRACT

A disintegrin and metalloprotease domain 33 (ADAM33) has been identified as an asthma susceptibility gene, which is associated with small-airway remodeling. However, the role of ADAM33 in the development of allergic airway inflammation is unclear. The present study used an established murine model of allergen-induced chronic airway inflammation, which was sensitized and then challenged by nebulized 2.5% ovalbumin (OVA) for 8 weeks (30 min/day, three times a week). The expression of ADAM33 mRNA detected by real time RT-PCR was significantly enhanced in the lung tissue of mice with OVA challenge, as compared with the group challenged with saline. This OVA-challenged model showed significant Th2-biased airway inflammation as well as airway remodeling with features of sub-epithelial fibrosis and mucus hyper-secretion. Furthermore, *in vitro* studies showed that IL-4 and IL-13 could significantly up-regulate the expression of ADAM33 mRNA in human fibroblasts in a concentration- and time-dependent manner as compared to normal controls. These results support the note that Th2 cytokines can up-regulate the expression of ADAM33 mRNA and ADAM33 may play an important role in the development of airway remodeling in allergen-induced chronic airway inflammation.

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1. Introduction

Asthma is a T-helper-cell 2 (Th2)-mediated chronic airway inflammation accompanied by bronchial hyperresponsiveness (BHR). The damage of epithelial cells, goblet cell hyperplasia, sub-epithelial fibrosis and smooth muscle hypertrophy and hyperplasia are involved in the development of BHR (Holgate et al., 2000). Activation of the epithelial–mesenchymal tropic unit in the airway, by interacting with cells which produce Th2 cytokines, is proposed to lead to the proliferation of fibroblasts and airway smooth muscle (ASM), contributing to BHR and airway remodeling (Davies et al., 2003). Multiple genes and environmental factors play important roles in the pathogenesis of asthma. A disintegrin and metalloprotease domain 33 (ADAM33), a recently

discovered ADAM family member, has been identified as an asthma susceptibility gene located on the short arm of chromosome 20 (20p13) through a genetic linkage and association study of families with asthma and BHR (Van Eerdewegh et al., 2002).

ADAM33 is a highly polymorphic gene containing more than 70 single nucleotide polymorphisms (SNPs) (Van Eerdewegh et al., 2002; Chae et al., 2003), and some of these SNPs are associated with BHR and asthma (Van Eerdewegh et al., 2002; Howard et al., 2003; Werner et al., 2004; Lee et al., 2004; Hirota et al., 2006). ADAM33 is preferentially expressed in fibroblasts and smooth muscle cells, rather than in epithelial cells and T cells or inflammatory leukocytes (Van Eerdewegh et al., 2002). The selective expression of ADAM33 strongly suggests that the alterations in its activity may be involved in small-airway remodeling. However, the relationship between the expression of ADAM33 in lung fibroblasts and the development of allergic airway inflammation has not been elucidated. In this study, we determined the expression of ADAM33 mRNA in an ovalbumin (OVA)-sensitized murine model of chronic airway inflammation and investigated the role of Th2 cytokines (IL-4 and IL-13) in the expression of ADAM33 mRNA in cultured human lung fibroblasts.

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2. Materials and methods

2.1. Animal

Female C57BL/6 mice were purchased from Shanghai Institute of the Chinese Academy of Medical Sciences. Mice, aging 6–8 weeks and weighing 16–18 g, were housed in Animal Center of Medical School of Fudan University at an environmentally controlled and specific pathogen-free conditions for 1 week prior to study, and for the duration of experiments. All procedures were reviewed and approved by the Institutional Review Board (IRB) of Fudan University.

2.2. Immunization procedures and exposure

Twenty female C57BL/6 mice were randomly divided into two groups ($n = 10/\text{group}$). In the OVA Group (allergen-induced chronic airway inflammation model), the mice were sensitized by an intraperitoneal injection of OVA 10 μg (Grade V, $\geq 98\%$ agarose gel electrophoresis, Sigma, A5503) precipitated with aluminum hydroxide (100 μg) on day 1 and 14. From day 21, mice were challenged by nebulized 2.5% OVA solution (30 min/day, three times a week for 8 weeks) in a whole-body inhalation exposure system. In the control group, the mice were sensitized and challenged by saline.

2.3. Bronchoalveolar lavage (BAL)

The mice were sacrificed at 24 h after the final OVA challenge, followed by BAL. Three aliquots of 1 ml phosphate buffered saline (PBS) were injected and withdrawn through the tracheal cannula. Following centrifugation, the supernatant was stored at -80°C for further measurement of IL-4 and IL-13 concentrations using enzyme-linked immunosorbent assay (ELISA). Total cell numbers were counted with a hemacytometer. The cytospin slides of BAL cells were prepared using a cytospin and stained with May–Grunwald–Giemsa. Differential cell counts were determined by light microscopy from a count of at least 200 cells.

2.4. Real-time RT-PCR

Total RNA from lung tissue was extracted using TRIzol reagent (Invitrogen). Reverse transcription was performed using 1 μg of total RNA and oligo (dT) primers in a 20- μl reaction according to the manufacturer's protocol. Real-time PCR was performed on the GeneAmp PCR System 9600 (PE Applied Biosystems) using FastStart DNA Master SYBR Green mixture (Roche Diagnostics) as a dsDNA-specific binding dye. 25 μl -reaction includes 5 $\times$ real time PCR buffer 5 μl , 5 $\times$ SYBR Green I 2.5 μl , FCD 1 μl , 10 mM dNTP 0.75 μl , 250 mM Mg^{2+} 0.3 μl , 5 U/ μl Hot Star (Ex Tag HS) 0.25 μl , 10 pmol/ μl forward and reverse primers 0.35 μl each, cDNA 2 μl , and DEPC- H_2O 12.5 μl . The primers sequences of ADAM33 (GenBank accession #NM.033615), IL-4, IL-13, IFN- γ and housekeeping gene hypoxanthineguanine phosphoribosyl transferase (HPRT) were synthesized by Shanghai Sangon Biological Engineering Technology Co, Ltd (Table 1).

The PCR was cycled 40 $\times$ after initial denaturation (95 $^\circ\text{C}$, 3 min), with the following parameters: denaturation, 95 $^\circ\text{C}$, 15 s; annealing, 60 $^\circ\text{C}$, 30 s; and extension, 79 $^\circ\text{C}$, 8 s. The threshold cycle (CT) was recorded for each sample to reflect the mRNA expression levels. A validation experiment confirmed the linear dependence of the CT value for HPRT at different known RNA concentrations. Specificity of the amplified products was assessed by melting curve analysis and gel electrophoresis. The ratio between the concentrations of target genes and HPRT was used to reflect the relative target gene expression levels.

Table 1

The sequences of forward primers and reverse primers of ADAM33 (GenBank accession #NM.033615), IL-4, IL-13, IFN- γ and housekeeping gene hypoxanthineguanine phosphoribosyl transferase (HPRT). All primers were synthesized for real-time RT-PCR.

	Sequences	Product size	
ADAM33	5'-GCG TTC GCA CCC TCC ATC TCT TGA-3'	368 bp	F
	5'-ATC GGG CTC TGG GGT TCC TAT GCT-3'		R
IL-4	5'-AGC TAG TTG TCA TCC TGC TC-3'	417 bp	F
	5'-GTG GCT CAG TAC TAC GAG TA-3'		R
IL-13	5'-ATG AGG AGA GAC CAT CCC TG-3'	108 bp	F
	5'-CAT CTC CCT TCC TCC TCA AC-3'		R
IFN- γ	5'-CTT TAA CAG CAG GCC AGA CA-3'	144 bp	F
	5'-GCG AGT TAT TTG TCA TTC GG-3'		R
HPRT	5'-ACA GCC AAC ACT GCT GAA AC-3'	272 bp	F
	5'-ACC CTC TGG TAG ATT GTC GC-3'		R

F: forward primer, R: reverse primer.

2.5. Lung histology

Left lungs were inflated with 4% paraformaldehyde–PBS solution to a pressure of 20 cm H_2O . After fixation for 24 h, the inflated lungs were embedded in paraffin. Thickness of 3 μm transverse sections were cut and stained with hematoxylin and eosin for histological assessment using light microscopy. Additional 3 μm sections were stained using Masson's Trichrome to demonstrate the presence of extracellular matrix (ECM), and periodic acid Schiff (PAS) stain to show the presence of mucus within goblet cells. Further 3 μm sections were prepared for immunohistochemical staining using a monoclonal antibody (DakoCytomation) against α -smooth muscle actin (α -SMA) to identify contractile elements.

Morphometric analysis of the lung sections was performed using a computerized image analysis system (Microscope: Olympus BX40; Camera: Panasonic MV-CP410 Video Camera; Software: IMS cell image analysis system). Morphometric quantification was calculated on the percentage of tissue area that was positively stained within regions of interest (one slide per mouse per stain). For each slide, five intact small airways with a diameter of 100–200 μm were randomly selected, and the mean value of these five small airways was used for each mouse. Images of all small airways were captured into a computer and saved for later analysis.

For sections stained with Masson's Trichrome and α -SMA, the region of interest was a 20 μm band immediately beneath the epithelium. This thickness was chosen because it was considered to include the ECM and contractile elements associated with airways, as reported previously (Leigh et al., 2002). Analysis of each image was initiated with the operator drawing a line along the basal border of the epithelium using a digital pen. This line did not cover any positively stained tissue associated with or beneath the airway epithelium. Thereafter, the macro software projected a second line 20 μm beneath the first line. In the case of sections with PAS, the operator added the second line along the inner border of the epithelium, and the region of interest became the area between the two lines. The area between these lines was calculated by the software, and regions within this area that were positively stained were identified based on previously determined settings of hue, saturation, and value.

2.6. Culture of human lung fibroblasts

Human fetal lung fibroblasts (MRC-5; lung, diploid, human, passage 7–12 cell line) were obtained from the American Type Culture Collection (Manassas, VA). Fibroblasts (2×10^6 cells) were cultured in 100-mm culture dishes. Before cytokine assay, fibroblasts (1×10^5 cells) were plated in 24-well tissue culture plates. Plated

Table 2

The number of total cells and differential cells in BALF were determined after repeated inhalation of OVA (OVA group) or saline (NS group) for 8 weeks in sensitized mice.

Treatment	Total ($\times 10^5$ /ml)	Monocytes ($\times 10^5$ /ml)	Eosinophils ($\times 10^5$ /ml)	Neutrophils ($\times 10^5$ /ml)	Lymphocytes ($\times 10^5$ /ml)
OVA	35.1 $\pm$ 6.8**	25.55 $\pm$ 4.83**	6.29 $\pm$ 1.27**	0.70 $\pm$ 0.46**	2.61 $\pm$ 0.62**
NS	12.8 $\pm$ 2.2	12.44 $\pm$ 2.03	0.08 $\pm$ 0.07	0.11 $\pm$ 0.11	0.20 $\pm$ 0.09

All data are expressed as means $\pm$ SEM, ** $P < 0.01$ compared with saline control mice ($n = 10$ /group).

fibroblasts were allowed to grow to confluence in regular growth medium (Dulbecco's modified Eagle's medium, DMEM) containing 10% fetal bovine serum (FBS); penicillin, 100 U/ml; streptomycin, 100 mg/ml and amphotericin B, 2.5 mg/ml. The same culture media was used when fibroblasts were stimulated with human recombinant IL-4 and human recombinant IL-13 (purchased from R&D). At specified points, cells were collected for RNA extraction.

2.7. Human ADAM33 expression

The expression of ADAM33 mRNA was detected using real-time RT-PCR. The reverse transcription and real-time RT-PCR were performed as described above. The primers of ADAM33 (GenBank #NM.025220) and housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were synthesized by Shanghai Sangon and were as follows: ADAM33, forward 5'-CTGAGAACTCTCATGAGC-3', reverse 5'-GACTCAGTCGAACCATAG-3', product size: 540 bp; GAPDH, forward 5'-GGTATCGTGAAGGACTCATGAC-3', reverse 5'-ATGCCAGTGAGCTTCCCGTTCAGC-3', product size: 188 bp. Δ CT (ADAM33 average CT-GAPDH average CT) was used to reflect the relative ADAM33 expression levels. To determine the effects of different stimuli on ADAM33 gene expression as compared with unstimulated cells, $\Delta\Delta$ CT was calculated ($\Delta\Delta$ CT = Δ CT stimulus – Δ CT unstimulated cells). The value of $2^{-\Delta\Delta$ CT} was calculated to demonstrate the fold changes of ADAM33 gene expression in stimulated cells as compared with unstimulated cells.

2.8. Data analysis

All data were analyzed using SPSS 10.0. Data are expressed as means $\pm$ SEM. Statistical analysis was determined by one-way ANOVA or paired t test. A value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Establishment of a murine model of allergen-induced chronic airway inflammation

A murine model of allergen-induced chronic airway inflammation was successfully established according to the method of Temelkovski et al. (1998). Briefly, C57BL/6 mice sensitized to OVA were challenged for 30 min/day on 3 days/week with aerosolized OVA for a period of up to 8 weeks. The number of total leukocytes, eosinophils and neutrophils were significantly increased in BALF obtained from OVA-exposed animals, as compared with that from saline-exposed ones (Table 2). The levels of IL-4 and IL-13 in BALF also were significantly increased in OVA group, as compared with NS group ($P < 0.01$, Table 3). The mRNA expressions of some cytokines in the lung tissue were detected by real-time RT-PCR. In OVA group, the relative quantities of IL-4 and IL-13 mRNA levels were significantly higher than those in NS group ($P < 0.01$, Fig. 1A). Although the expression of IFN- γ was also slightly increased in OVA group, the ratios of IL-4/IFN- γ and IL-13/IFN- γ were greatly increased ($P < 0.05$, Fig. 1B). The results indicated that Th2 cytokines-bias immune response was induced in this model.

In addition to Th2 immune response, airway remodeling was also found in this model (Fig. 2A). In allergen-induced chronic

airway inflammation murine model, HE staining demonstrated marked peribronchial inflammatory infiltrates, Masson Trichrome staining revealed marked sub-epithelial collagen deposition, and PAS staining showed mucus metaplasia. The increased contractile elements by α -SMA staining were also evident in the airways of OVA group compared with control saline mice. The quantitative analysis showed that the positive areas percentages of Masson's, PAS, and α -SMA staining were significantly higher in OVA group than those in NS group ($P < 0.01$, Fig. 2B).

3.2. The expression of ADAM33 mRNA increased in the lung tissue of chronic asthmatic mice model

After successfully established a murine model of chronic airway inflammation, we wanted to know whether ADAM33 mRNA expression would change after repeated exposures to OVA. Real-time RT-PCR was performed to confirm the changes of ADAM33 at mRNA level in chronic airway inflammation mice. The relative quantity of ADAM33 mRNA in lung tissue of OVA challenged mice was $(1.41 \pm 0.93) \times 10^{-2}$, which was significantly higher than that of saline control mice $((0.37 \pm 0.31) \times 10^{-2}$, $P < 0.01$, Fig. 3). The result showed that the expression of ADAM33 mRNA could up-regulate in the allergen-induced chronic airway inflammation mice.

3.3. IL-4 and IL-13 could induce the up-regulation of ADAM33 mRNA expression on fibroblasts

To investigate the mechanism of increased expression of ADAM33 in allergen-induced chronic airway inflammation mice, human fibroblasts (MRC-5) on which ADAM33 is specifically expressed were cultured either alone or in the presence of IL-4 or IL-13. MRC-5 cells were stimulated with different doses of IL-4 or IL-13 (0, 0.1, 1 and 10 ng/ml) to investigate dose-related responses. After incubation for 24 h, the expression of ADAM33 mRNA was detected by real-time RT-PCR. The results revealed that IL-4 and IL-13 enhanced the expression of ADAM33 mRNA in a dose-dependent manner (Fig. 4A). MRC-5 cells were then incubated with saline (control), IL-4 (10 ng/ml) and IL-13 (10 ng/ml) for 6, 12, 24, and 48 h to find out the dynamic alterations. The expression of ADAM33 mRNA began to increase on 6 h, peak on 24 h, and decline on 48 h after IL-4 and IL-13 stimulation (Fig. 4B). The additive effect between IL-4 and IL-13 was also observed. The ADAM33 mRNA expression on MRC-5 cells stimulated with both IL-4 (10 ng/ml) and IL-13 (10 ng/ml) was significantly higher than that stimulated with IL-4 or IL-13 alone (Fig. 5).

Table 3

The concentration of IL-4 and IL-13 in BALF was determined by ELISA after repeated inhalation of OVA (OVA group) or saline (NS group) for 8 weeks in sensitized mice.

Treatment	IL-4 (pg/ml)	IL-13 (pg/ml)
OVA	279.3 $\pm$ 52.7**	4015 $\pm$ 361**
NS	10.3 $\pm$ 7.4	1594 $\pm$ 185

All data are expressed as means $\pm$ SEM, ** $P < 0.01$ compared with saline control mice ($n = 10$ /group).

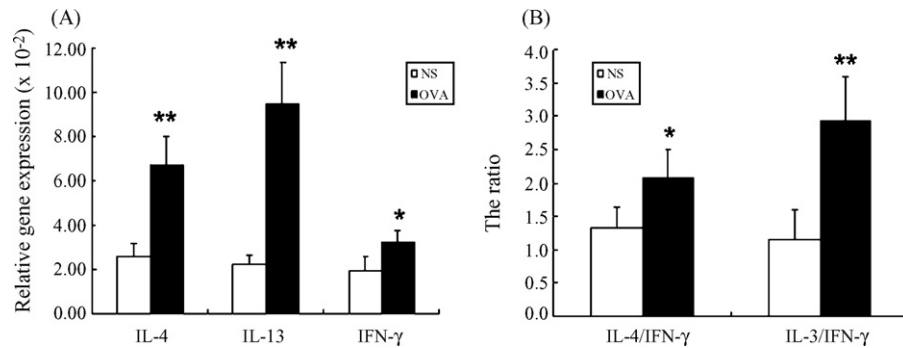


Fig. 1. Repeated inhalation of OVA in sensitized mice leads to products of Th2 cytokines. C57BL/6 mice sensitized to ovalbumin were challenged for 30 min/day on 3 days/week with aerosolized ovalbumin for 8 weeks. The mRNA expressions of some cytokines in the lung tissue were detected by real-time RT-PCR. (A) The relative quantities of IL-4, IL-13 and IFN-γ mRNA levels in the lung tissue were calculated, which were normalized to the level of HPRT that served as an internal control for the amount of RNA used in each reaction. (B) The ratios of IL-4/IFN-γ and IL-13/IFN-γ were calculated. All data are expressed as means ± SEM, **P* < 0.05 and ***P* < 0.01 compared with saline control mice (*n* = 10/group).

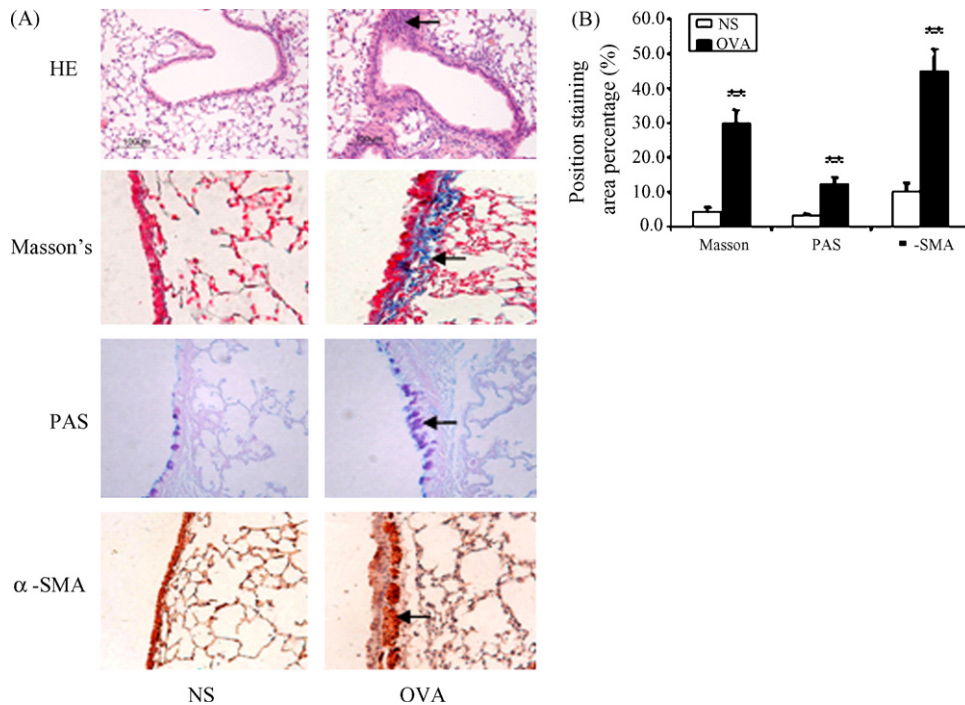


Fig. 2. The changes of airway remodeling in a murine model of chronic asthma. (A) Repeated inhalation of OVA for 8 weeks in sensitized mice leads to significant chronic peribronchial inflammation characterized by inflammatory cells infiltration (hematoxylin and eosin staining, indicated by arrow, 20×) and airway remodeling characterized by subepithelial fibrosis (Masson Trichrome staining, blue, indicated by arrow, 40×), mucus hyper-secretion (PAS staining, magenta, indicated by arrow, 40×) and airway smooth muscle thickening (α-SMA staining, brown, indicated by arrow, 40×). (B) Morphometric changes in the airway following chronic exposure to OVA. Staining was assessed using morphometry for sub-epithelial fibrosis (Masson's Trichrome), contractile elements (α-SMA) in the 20 μm region beneath epithelium and mucus (PAS) in the epithelial portion of the airway wall. Data are expressed as the percentage of the region of interest that was positively stained. ***P* < 0.01 compared with saline control mice (*n* = 10/group).

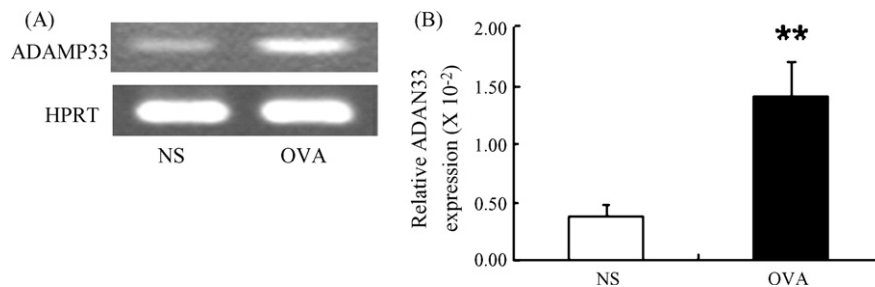


Fig. 3. Up-regulation of ADAM33 at mRNA level in OVA inhalation mice compared with saline control mice. C57BL/6 mice sensitized to ovalbumin were challenged for 30 min/day on 3 days/week with aerosolized ovalbumin for 8 weeks. The mRNA expressions of ADAM33 in the lung tissue were detected. (A) The expression of ADAM33 at mRNA level was measured by gel electrophoresis after real-time RT-PCR. (B) Real-time RT-PCR was performed to confirm up-regulation of ADAM33 at mRNA level in OVA inhalation mice compared with saline control mice. ADAM33 mRNA levels were normalized to the level of HPRT that served as an internal control for the amount of RNA used in each reaction. Data are expressed as means ± SEM, ***P* < 0.01 compared with saline control mice (*n* = 10/group).

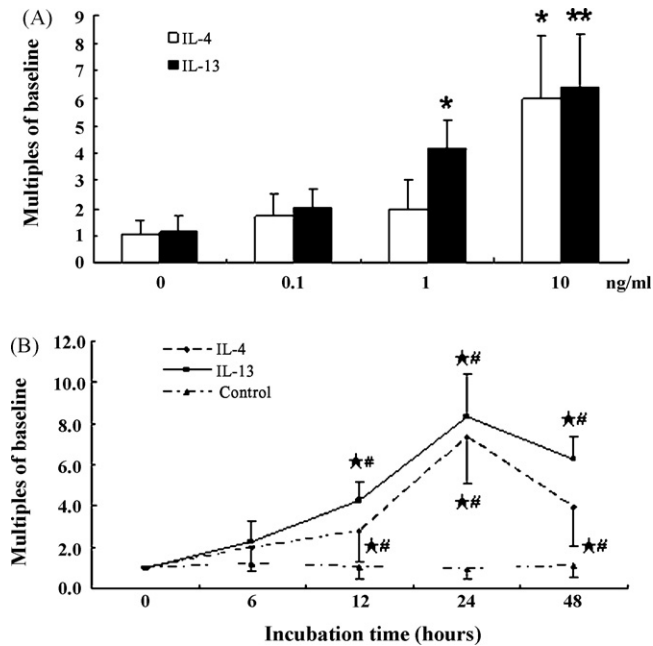


Fig. 4. IL-4 and IL-13 could up-regulate the expression of ADAM33 mRNA on the human lung fibroblasts in a dose and time dependent manner. Human lung fibroblasts (MRC-5) were cultured either alone or in the presence of IL-4 and IL-13 from 6 to 48 h and the expression of ADAM33 mRNA was examined by real-time RT-PCR. (A) Concentration dependent effects of IL-4 and IL-13 on ADAM33 mRNA expression on MRC-5. The cells were incubated with various concentrations of IL-4 and IL-13 for 24 h. Data are expressed as means $\pm$ SEM ($n=6$), * $P<0.05$; ** $P<0.01$ vs untreated cells. (B) Time-dependent effects of IL-4 and IL-13 on ADAM33 mRNA expression. MRC-5 cells were incubated with saline (control), IL-4 (10 ng/ml) and IL-13 (10 ng/ml) from 6 to 48 h and the expression of ADAM33 mRNA was examined by real-time RT-PCR. At the zero time point, the data point is one-fold. Data are expressed as means $\pm$ SEM ($n=6$), * $P<0.05$ compared with zero time point; # $P<0.05$ compared with control cells at the same time point.

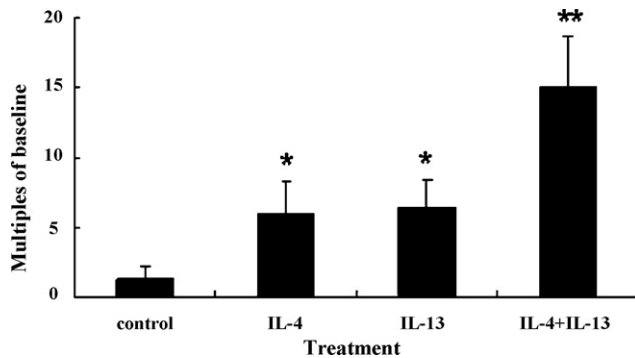


Fig. 5. Additive effects of IL-4 and IL-13 on the ADAM33 mRNA expression on MRC-5. MRC-5 cells were incubated with saline (control), IL-4 (10 ng/ml) and IL-13 (10 ng/ml) alone or together for 24 h, then the expression of ADAM33 mRNA was analyzed by real-time RT-PCR. Data are expressed as means $\pm$ SEM ($n=6$), * $P<0.05$ compared with untreated cells; ** $P<0.05$ compared with untreated cells and other groups.

4. Discussion

A murine model of allergen-induced chronic airway inflammation was successfully established in the present study, according to the method of Temelkovski et al. (1998). We found that the ADAM33 mRNA expression was significantly enhanced in the lung tissue from mice with repeated allergen challenge, which induced both Th2-bias airway inflammation and airway remodeling, with features of sub-epithelial fibrosis and mucus hyper-secretion. In vitro, we found that the ADAM33 mRNA was slightly expressed in

unstimulated human fibroblasts. However, the Th2 cytokines (IL-4 and IL-13) could up-regulated the expression of ADAM33 mRNA in the human fibroblasts in a concentration- and time-dependent manner. These results indicate that ADAM33 may play an important role in the pathogenesis of airway remodeling in chronic asthma, related with Th2 mediated inflammation.

After the ADAM33 gene was first proved to be associated with asthma (Van Eerdewegh et al., 2002), the correlations between ADAM33 gene and asthma have been observed in diverse populations, such as Dutch, American, German, Korean and Japanese asthma population (Howard et al., 2003; Werner et al., 2004; Lee et al., 2004; Hirota et al., 2006). Recent studies demonstrated that the SNPs of the ADAM33 gene were also associated with asthma in Chinese Han population (Qiu et al., 2007; Su et al., 2008). In addition to asthma susceptibility, polymorphisms of ADAM33 gene were found to be associated with the development of lung dysfunction (Jongepier et al., 2004). ADAM33 has been shown to be expressed in airway fibroblasts and ASM cells, suggesting a role in modifying the function of these cells, e.g. proliferation, differentiation and migration. So the alterations of its activity might be involved in airway remodeling.

There are conflicting findings on changes of ADAM33 production or activity in asthma. Some studies found that ADAM33 expression quantified by RT-PCR wasn't increased in bronchial biopsy specimens from subjects with mild or severe asthma compared with normal subjects (Haitchi et al., 2005; Yang et al., 2008). However, other studies found that the levels of ADAM33 protein and mRNA were increased in BALF or bronchial biopsies from subjects with asthma compared with normal subjects (Lee et al., 2006; Ito et al., 2007; Foley et al., 2007). Yang et al. (2008) thought that one possible explanation for the difference between the published data lies in biopsy sampling and tissue heterogeneity. Because ADAM33 is expressed in smooth muscle cells and not in epithelial cells, the level of expression will reflect the proportion of smooth muscle in each biopsy specimen. Another study demonstrated that ADAM33-null mice did not show allergen-induced airway hyperreactivity, IgE production, mucus metaplasia, and airway inflammation (Chen et al., 2006). In contrast, our study found that the expression of ADAM33 mRNA in the lungs was significantly higher after repeated allergen challenge. The inconsistency of these two studies might be due to different strategies of model induction, e.g. mice were intranasally challenged with OVA only for days in the early study (Chen et al., 2006), while mice were repeated inhaled OVA for 8 weeks in our study.

We further investigated the possible mechanisms of up-regulation of ADAM33 mRNA. IL-4 and IL-13 are Th2 cytokines that play important roles in allergic inflammation and airway remodeling. It has been shown that human lung fibroblasts express functional receptors for IL-4 and IL-13 and respond to these cytokines (Doucet et al., 1998). IL-13 was suggested to be a potent stimulus for fibroblast proliferation and collagen production (Lee et al., 2001). Thus we hypothesized that IL-4 and IL-13 might regulate the expression of ADAM33 via the receptors. ADAM33 has at least two isoforms (α and β) expressed in different lung cell types (Yoshinaka et al., 2002). The β isoform lacks 26 amino acids (exon Q) between a cysteine-rich domain and an EGF-like domain. This lack of maturation coupled with localization to the endoplasmic reticulum suggests that the isoform β exerts a dominant negative effect on protease activity (Umland et al., 2004). The products of real-time RT-PCR in the present study included both isoforms α and β , since the sense primer started in exons U and the antisense primer started in exon V. We found that human lung fibroblasts expressed low levels of ADAM33 mRNA, and IL-4 and IL-13 could increase the expression of ADAM33 mRNA in a dose- and time-dependent manner. Moreover, IL-4 and IL-13 interacted additively in inducing the expression of ADAM33 mRNA in human lung fibroblasts.

Our data were similar in part to the previous findings (Foley et al., 2007). They found that stimulation of normal human bronchial epithelial cells (NHBE) with 10 ng/ml IL-4 led to induction of ADAM33 α isoforms mRNA at 24 h with a maximal effect, but no induction of the ADAM33 expression occurred after stimulation of with IL-13 (Foley et al., 2007). However, another study found that neither IL-4 nor IL-13 had any effects on ADAM33 (both α and β) mRNA expression in ASM cells, but they did report 30–40% inhibition of ADAM33 expression by interferon- γ (IFN- γ) (Ito et al., 2007). Different cell types used in these studies could explain the discrepancy in these results.

In summary, the present study demonstrates that the expression of ADAM33 mRNA enhances in the lungs of a murine model of allergen-induced chronic airway inflammation. The increase of ADAM33 mRNA expression might be due to the stimulation of IL-4 and IL-13 on lung fibroblasts. The function of ADAM33 may contribute to airway remodeling in chronic processes.

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