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Cysteinyl leukotrienes regulate TGF- β_1 and collagen production by bronchial fibroblasts obtained from asthmatic subjects[☆]

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SUMMARY

Background: Cysteinyl leukotrienes (CysLTs) play an important role in airway inflammation in asthma but their role in airway remodeling is not completely known.

Methods: CysLTs receptors and procollagen I(α_1) mRNA were determined by qPCR. Procollagen protein production was measured by RIA and TGF- β_1 expression was determined by ELISA. TGF- β receptor expression was assessed by western blots.

Results: CysLT1R, TGF- β -R1 and active TGF- β_1 are highly expressed in cells from asthmatics compared to normal controls. LTD₄ increased significantly procollagen I(α_1) mRNA and protein expression in fibroblasts from asthmatics. This increase was blocked by CysLTs receptor antagonist. LTD₄ increased significantly mRNA expression of TGF- β_1 and active form production in fibroblasts from asthmatics. Inhibition of TGF- β_1 signaling blocked LTD₄-induced procollagen I(α_1) expression.

Conclusions: Fibroblasts from asthmatic subjects express high level of CysLT1R. LTD₄ regulates procollagen I(α_1) transcription in fibroblasts derived from asthmatic patients by modulating TGF- β_1 expression. This suggests that CysLTs may play a role in regulating collagen deposition in asthma.

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1. Introduction

Allergic asthma is characterized by episodes of chronic airway inflammation following allergen challenge [1]. Subsequent healing and constant cycles of repair and damage is thought to lead to nonreversible airflow obstruction. In asthma, the bronchial mucosa undergoes major structural alterations characterized by epithelial desquamation and subepithelial fibrosis [2]. This deposition of interstitial collagen underneath the basement membrane is a characteristic feature of asthma. This seems to be due to the activation of subepithelial fibroblasts. These cells acquire the myofibroblast phenotype. There is a correlation between the number of myofibroblasts in the bronchial mucosa and the degree of subepithelial fibrosis observed in asthma [3]. These fibroblasts increase in number after an allergen challenge [4].

Cysteinyl leukotrienes (CysLTs) are potent pro-inflammatory lipid mediators secreted by eosinophils and mast cells and they

act on a wide variety of cell types. They are important contributors in the pathophysiology of inflammatory and allergic diseases such as asthma [5,6]. CysLTs are involved in recruitment of inflammatory cells to the bronchial mucosa, in bronchoconstriction and mucous hypersecretion [7,8]. They signal through two G-protein-coupled receptors named CysLT1R and CysLT2R [9]. The CysLT1R receptor is expressed on human lung smooth muscle, eosinophils, macrophages and peripheral blood leukocytes [10]. The receptor is also expressed in human nasal mucosa [11]. CysLT1R is the target of anti-asthmatic drugs such as Montelukast (MK-0476) that have both anti-bronchoconstrictive and anti-inflammatory actions [12,13].

In vitro and animal model studies have revealed that CysLTs may play a role in the remodeling process observed in asthma. They regulate smooth muscle cell proliferation induced by growth factors and migration [14,15]. TGF- β_1 , IL-13 and IFN- γ upregulate the expression of CysLT1R in bronchial smooth muscle cells [16]. Leukotriene-deficient mice are protected from pulmonary fibrosis [17]. Furthermore, targeted disruption of phospholipase A₂ attenuates bleomycin-induced pulmonary fibrosis in mice [18]. In ovalbumin-challenged mice, CysLT1R antagonist MK-0476 reduces collagen deposition in the lung [19]. It has been shown that LTD₄ in combination with TGF- β_1 stimulates collagen production by human fetal lung fibroblasts [20]. However, little is known about the effect of CysLTs on airway remodeling in human asthma. In this study, we have investigated the effect of CysLTs

Abbreviations: CysLTs, Cysteinyl leukotrienes; MK-0476, Montelukast

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and their antagonist on TGF- β_1 and collagen production in human bronchial fibroblasts derived from asthmatic subjects and healthy controls.

2. Methods

2.1. Bronchial fibroblast cell culture

Primary fibroblasts were isolated from bronchial biopsies of asthmatic ($n=8$) and healthy ($n=8$) subjects. The asthmatic subjects fulfilling the American Thoracic Society criteria for asthma [21] were recruited from the Asthma Clinic at Institut Universitaire de Cardiologie et de Pneumologie de Québec. They used only an inhaled β_2 -agonist on demand. None used inhaled or systemic corticosteroids. All asthmatic patients were atopic non-smoker (mean age=22 years). Healthy subjects (mean age=26 years) were non-atopic non-smoker with no history of asthma or respiratory and systemic diseases. Characteristics of the subjects are summarized in Table 1. All subjects underwent bronchoscopy with bronchial biopsies to isolate bronchial fibroblasts as described previously [22]. The study was approved by the ethics Committee at the Institut Universitaire de Cardiologie et de Pneumologie de Québec and written informed consent was obtained from all subjects before entry into the study.

Isolated fibroblasts were characterized by immunofluorescence and flow cytometry using a mouse anti-vimentin and a mouse anti-human fibroblast antigen Ab-1 antibody (Calbiochem, San Diego, CA, USA) that shows no cross-reactivity with epithelial cells, endothelial cells, smooth muscle cells or other cell types. This identification confirmed the purity of bronchial fibroblast cell culture as described previously [23,24].

2.2. Real-time quantitative polymerase chain reaction

Total RNA was extracted from stimulated fibroblasts using the illustra RNeasy Mini RNA Isolation Kit (GE Healthcare, Buckinghamshire, UK) following the manufacturer's protocol. Concentrations of total RNA were measured by a fluorescent dye with the Ribogreen RNA quantification reagent (Molecular Probes Inc., Eugene, OR, USA). Total cDNA was synthesized using M-MLV RT (Invitrogen, Burlington, ON, Canada). Procollagen I(α_1), and GAPDH were amplified by PCR using primers previously described [25]. Primers for CysLT1R were: 5'-CGGGATCCGAT-GAAACAGGAAATC-3' as sense and 5'-CCGGAATTCAATGGGTT-TAACTATAC-3' as antisense with an annealing temperature of 52.5 °C. Primers for CysLT2R were 5'-CCAGATTAGCATGAAAACG-3' as sense and 5'-CCTTCAGCAATAACAACAGC-3' as antisense with an annealing temperature of 56 °C. Primers for TGF- β_1 were 5'-GCCCTGGACACCAACTATT-35 as sense and 5'-AGGCTCCAAATG-TAGGGG-3' as antisense with an annealing temperature of 60 °C. Real-time quantitative polymerase chain reactions were

performed using the B-R-SYBRGreen Supermix (Quanta Bioscience). Data were acquired and analyzed with the computer software Opticon Monitor (version 2.02.24). Normalized expressions were calculated using the calculated efficiency of each PCR reaction with a cDNA standard curve. Ratios were calculated using the absolute quantity of the tested gene compared to GAPDH for each condition.

2.3. Intracellular calcium determination

Bronchial fibroblasts were plated at 1.5×10^4 cells in 2.5 cm petri dishes and incubated with 2.5 μ M Fura-2-am (Molecular Probes Inc., Eugene, OR, USA) for 45 min at ambient temperature. Cells were rinsed three times with 1.8 mM calcium buffer and pre-treated or not with MK-0476 10^{-8} M one hour prior to experiment. Fluorescence was recorded every 4 s for 8 min to ensure calcium stability in non-stimulated cells before stimulation with 100 nM LTD $_4$ (Cayman Chemicals, Ann Arbor, MI, USA). Changes in calcium concentration were followed by Fura-2 fluorescence using the Imagemaster system (Photon Technology International, Mommouth Junction, NJ, USA) coupled to a Leica DM IRB Fluorescence microscope (40 $\times$) (Leica Canada Inc., St-Laurent, QC, Canada). Excitation wavelengths were 353 and 374 nm with a correction for emission wavelength at 510 nm. The ratio between the fluorescence provoked by the 353 and the 374 nm excitation wavelengths was calculated and used as an intracellular concentration indicator.

2.4. Western blots

Total protein extracts were prepared from cultured fibroblasts. Proteins in reducing sample buffer were separated on 10% acrylamide SDS-PAGE gels. Proteins were transferred to PVDF membranes and visualized by soaking it in a 0.5% Rouge Ponceau, 1% acetic acid solution to confirm equal protein loading. TGF- β -R1, TGF- β -R2 (1:500, Santa Cruz biotechnology, Santa Cruz, CA, USA) or β -Actin (1:10 000, Sigma Chemicals, St Louis, MO, USA) antibodies were incubated overnight. Immunodetection was performed using a chemiluminescent kit (Millipore, Billerica, MA, USA) followed by autoradiography on X-Omat blue films.

2.5. Cell stimulation

Bronchial fibroblasts from healthy or asthmatic subjects were plated at 2×10^5 cells/well in 12 well plates (Costar, NY, USA) in 10% FBS supplemented Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Burlington, ON, Canada) and allowed to adhere overnight before stimulation. Fibroblasts were then washed and stimulated for 2 to 12 h with LTD $_4$ (Cayman Chemicals, Ann Arbor, MI, USA) at 0, 10, 100 or 1000 nM, in presence or absence of different concentrations of MK-0476 (10^{-6} or 10^{-8} M) (Merck Frosst, Kirkland, QC, Canada). In some experiments, cells were pre-treated with 10 μ M of SB431542, an inhibitor of TGF- β receptor (Calbiochem, San Diego, CA, USA) one hour prior to stimulation.

2.6. Measurement of procollagen I protein production in culture supernatant

Procollagen type I was measured with a radioimmunoassay kit (Orion Diagnostica, Espoo, Finland) as we previously reported [23]. Results obtained were expressed as μ g/L and normalized to cell number.

Table 1
Subjects' characteristics.

	Controls ($n=6$)	Asthma ($n=29$)
Age (years)	26.4 $\pm$ 1.5	22.5 $\pm$ 1.4
Gender M/F	4/2	3/3
Atopy (n)	0	6
FEV $_1$ (% predicted)	98.2 $\pm$ 5.1	85 $\pm$ 3.1
PC $_{20}$ (mg/ml)	83.8 $\pm$ 16.1	2.5 $\pm$ 1.1
Asthma medication	–	Bd (prn)

FEV $_1$: forced expiratory volume in one second; PC $_{20}$: provocative concentration of methacholine inducing a 20% fall in FEV $_1$; Bd (prn): inhaled bronchodilator as needed.

2.7. Total and active TGF- β 1 measurements

Total TGF- β 1 was measured in cell culture supernatants by ELISA using a commercial kit (R&D systems, Minneapolis, MN, USA) following the manufacturer's instructions. Cell culture supernatants were acidified with 1 N HCl for 10 min at ambient temperature and neutralized with 1.2 N NaOH/0.5 M HEPES prior to testing to ensure activation of total TGF- β 1 into immunoreactive form. Plates were read at 450 nm with a wavelength correction at 550 nm using a microplate reader (Molecular Devices Corp. CA, USA). The minimum detectable level of TGF- β 1 was less than 5 pg/ml.

Active form was measured using Mink lung epithelial cells kindly provided by Dr Rifkin (New York University Medical Center, NY, USA). These cells have been stably transfected with an 800 bp fragment (–799 to +71) of the 5' end of the human plasminogen activator inhibitor-1 (PAI-1) gene fused to the firefly luciferase reporter gene in a p19LUC-based vector containing the neomycin-resistance gene from pMAMneo. Transfected MLECs were maintained in DMEM supplemented with 10% FBS, 2 mM L-glutamine and 200 μ g/ml G418 (Invitrogen, Burlington, ON, Canada). Active TGF- β 1 containing supernatant samples were added directly to 1.6×10^4 cells and incubated for 16 h. Cells were then washed and lysed. Luciferase activity was measured with a Dynex 96-well plate luminometer (Dynex Technologies, Chantilly, VA, USA).

2.8. Statistical analysis

All experiments in this study were performed at least three times. To compare groups and conditions, the data were analyzed using a crossed-nested design. When interactions between groups and conditions were significant, each group was analyzed using a randomized block design. A posteriori multiple comparisons were performed using Tukey–Kramer test. Mean values $\pm$ SEM of quantitative variables were used as representative measures. All reported *p* values were two-sided and were declared significant at 0.05 levels. All analyses were conducted using the statistical package SAS.

3. Results

3.1. CysLTs receptors expression in bronchial fibroblasts.

To determine CysLT receptors expression profile in bronchial fibroblasts, we performed real-time PCR on cells isolated from asthmatic subjects or healthy controls. Bronchial fibroblasts isolated from asthmatic subjects overexpressed CysLT1R (ratio: 0.112 ± 0.026) compared to normal controls (ratio: 0.036 ± 0.016 , $p=0.02$) (Fig. 1). mRNA expression of CysLT2R is very low compared to CysLT1R and no difference was found between asthmatic and healthy controls.

3.2. Intracellular calcium modulation by LTD₄.

Calcium internalization was used as a measure to ensure CysLT receptor activity. We observed a significant and instantaneous intracellular calcium rise in bronchial fibroblasts. Pretreatment with MK-0476 one hour prior to experiment completely inhibited calcium mobilization induced by LTD₄. No difference was observed in intracellular calcium rise after LTD₄ stimulation between normal (Fig. 2A) and asthmatic cells (Fig. 2B).

3.3. TGF- β receptors expression and baseline TGF- β 1 production.

TGF- β 1 is a well-known inducer of collagen expression. TGF- β R1 and TGF- β -R2 receptor protein expressions were determined

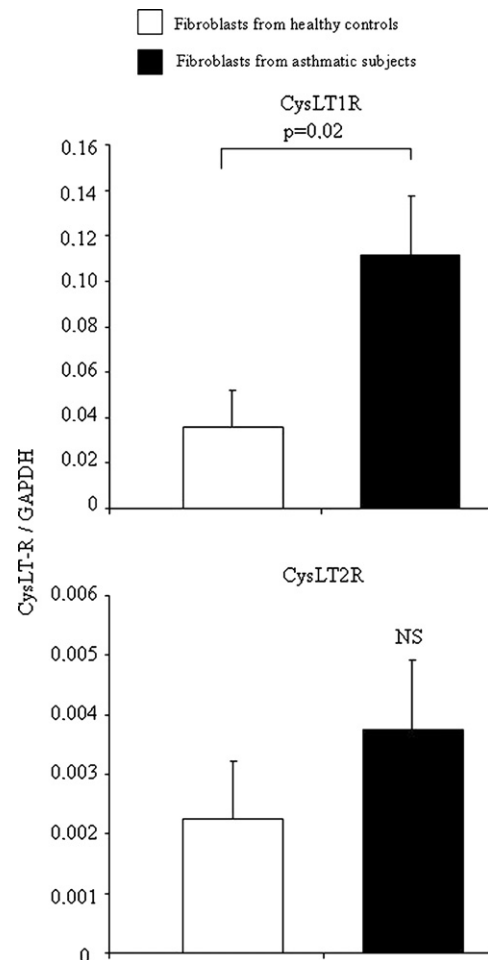


Fig. 1. CysLTs receptors expression in bronchial fibroblasts. Real-Time PCR analysis of CysLT1R and CysLT2R mRNA in bronchial fibroblasts obtained from normal controls or mild asthmatic subjects.

by western blots (Fig. 3A). Bronchial fibroblasts expressed both TGF- β -R1 and TGF- β -R2. Semi-quantitative analyses were performed and showed that bronchial fibroblasts isolated from asthmatic subjects overexpressed TGF- β -R1 (2.74 ± 0.31 in asthmatics versus 0.99 ± 0.14 arbitrary units, $p=0.008$) but not TGF- β -R2 (1.12 ± 0.08 versus 1.04 ± 0.12 arbitrary units, $p=0.31$).

There was no difference in baseline production of total form of TGF- β 1 by fibroblasts from normal and asthmatic subjects (3126 ± 583 pg/ml and 2735 ± 469 pg/ml, respectively, $p=0.61$) (Fig. 3B). However, we measured the active form of TGF- β 1 by a bioassay using mink lung epithelial cells transfected with a plasmid containing the luciferase reporter gene under the control of the PAI-1 promoter. Fig. 3B shows that the active form of TGF- β 1 is higher in fibroblasts isolated from asthmatic subjects compared to healthy controls (2.233 ± 0.461 pg/ml vs. 3.527 ± 0.223 pg/ml, $p=0.03$).

3.4. Effect of LTD₄ on TGF- β 1 mRNA production and protein production.

Fig. 4A shows that LTD₄ significantly increased TGF- β 1 mRNA production in asthmatic fibroblasts (0.024 ± 0.003 TGF- β 1/GAPDH ratio compared to 0.015 ± 0.002 TGF- β 1/GAPDH ratio in unstimulated controls, $p=0.04$) and this effect can be blocked by MK-0476 (0.012 ± 0.001 TGF- β 1/GAPDH ratio, $p=0.02$) while no significant effect was observed in fibroblasts obtained from healthy subjects (data not shown). Fig. 4B showed that increased mRNA

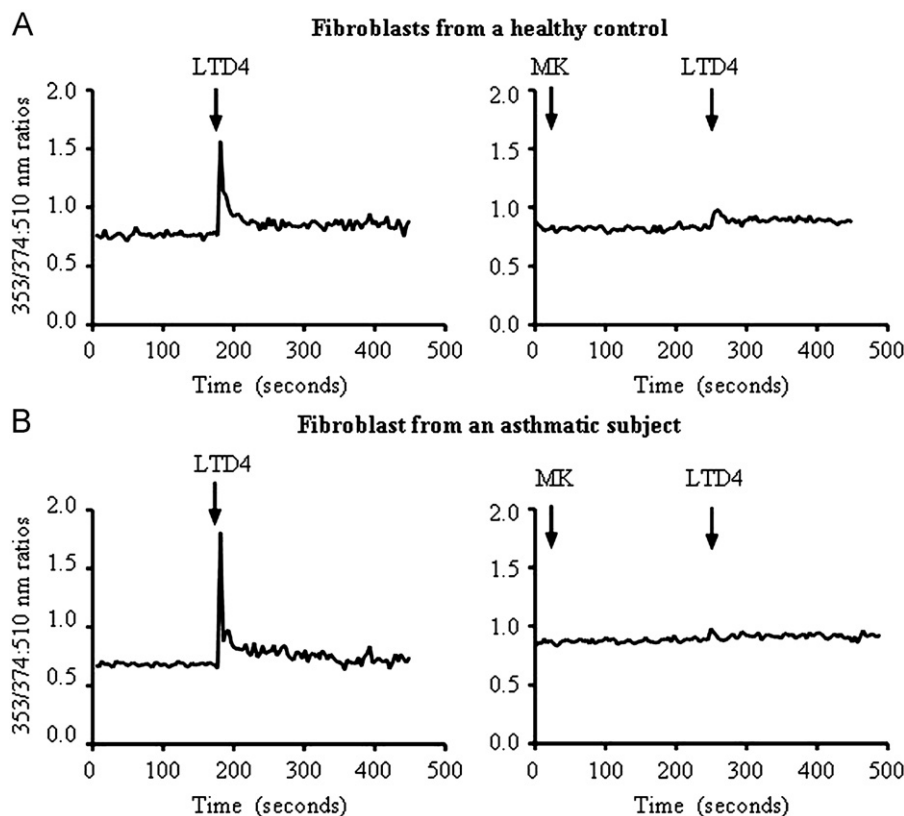


Fig. 2. Intracellular calcium levels after stimulation with LTD₄ in bronchial fibroblasts cells isolated from normal (A) or asthmatic subjects (B) were pre-treated or not with MK-0476 (MK; 10⁻⁸ M) for 1 h and intracellular calcium levels were measured. LTD₄ stimulation (100 nM) occurred at the time indicated by the arrow. A representative data from one asthmatic and one control is shown.

production is accompanied with an increase in active form of TGF- β_1 ($126 \pm 9\%$, $p=0.02$). CysLTs antagonist MK-0476 inhibited LTD₄-induced active TGF- β_1 production ($90 \pm 10\%$ and $99 \pm 8\%$, $p=0.01$). We have also tested the effect of CysLTs on the total protein production of TGF- β_1 in fibroblasts from asthmatics by ELISA. LTD₄ increased total TGF- β_1 protein expression ($127 \pm 9\%$, $p=0.02$) and this increase was blocked by MK-0476 ($102 \pm 10\%$, $p=0.04$) (data not shown).

3.5. Procollagen I(α_1) mRNA modulation by LTD₄.

To determine whether CysLTs modulate procollagen I(α_1) mRNA expression, bronchial fibroblasts were stimulated with different concentrations of LTD₄ (0, 10, 100 or 1000 nM) and tested by real-time PCR using procollagen I(α_1) specific primers. A dose response and time course studies showed that the optimal dose of LTD₄ was 100 nM and the peak of procollagen I(α_1) mRNA expression was attained after 2 h of stimulation. Fig. 5A shows that LTD₄ has no effect on procollagen I(α_1) mRNA expression in normal cells. However, LTD₄ increased procollagen I(α_1) mRNA expression in asthmatic fibroblasts at 100 nM (collagen/GAPDH ratios of 2.708 ± 0.28 vs. 1.52 ± 0.22 , $p=0.05$). CysLT receptor antagonist MK-0476 at all doses tested (10⁻⁶ and 10⁻⁸ M) was able to inhibit CysLTs-induced procollagen I(α_1) mRNA increase in asthmatic cells. Fig. 5B shows that MK-0476 at 10⁻⁸ M decreased LTD₄-induced collagen to baseline value (1.31 ± 0.31 , $p=0.05$ and 1.56 ± 0.17 , $p=0.05$ collagen/GAPDH ratio, respectively). CysLTs receptor antagonists alone had no significant effect on procollagen I(α_1) mRNA production by bronchial fibroblasts.

To assess the effect of leukotrienes and their antagonists on collagen protein production, we measured procollagen I protein secretion in cell culture supernatants of stimulated fibroblasts. At

baseline, there was no difference in baseline procollagen I production by bronchial fibroblasts from normal or asthmatic subjects (91.0 ± 12.3 $\mu\text{g/L}$ and 106.5 ± 10.0 $\mu\text{g/L}$ respectively, $p=0.19$). Cells were stimulated for 48 h with LTD₄ and/or MK-0476. Procollagen I concentrations were normalized for cell number prior to analysis. Fig. 5C shows that LTD₄ induced procollagen I production in cell culture supernatants in fibroblasts from asthmatics (131.8 ± 17.6 $\mu\text{g/L}$, $p=0.02$). This production is inhibited by MK-0476 (112.1 ± 15.7 $\mu\text{g/L}$, $p=0.02$). The effect on fibroblasts from healthy controls is modest and not statistically significant.

3.6. Effect of TGF- β_1 inhibition on LTD₄-induced procollagen I(α_1) production.

To determine whether TGF- β_1 is involved in LTD₄-induced procollagen I(α_1) production, we inhibited the TGF- β receptor using an ALK5 inhibitor, SB431542, before stimulation with LTD₄ for 2 h. As shown in Fig. 6, TGF- β_1 inhibition abolished LTD₄-induced procollagen I(α_1) mRNA expression in asthmatic cells (0.386 ± 0.061 collagen/GAPDH ratio compared to 0.058 ± 0.007 collagen/GAPDH ratio in LTD₄-stimulated cells, $p=0.04$) suggesting that LTD₄ affected procollagen I(α_1) mRNA production directly through the action of TGF- β_1 .

4. Discussion

In this study, we showed that bronchial fibroblasts derived from asthmatic airways express high level of CysLT1R compared to cells derived from healthy controls. Moreover, LTD₄ significantly induced both procollagen I(α_1) and TGF- β_1 mRNA and

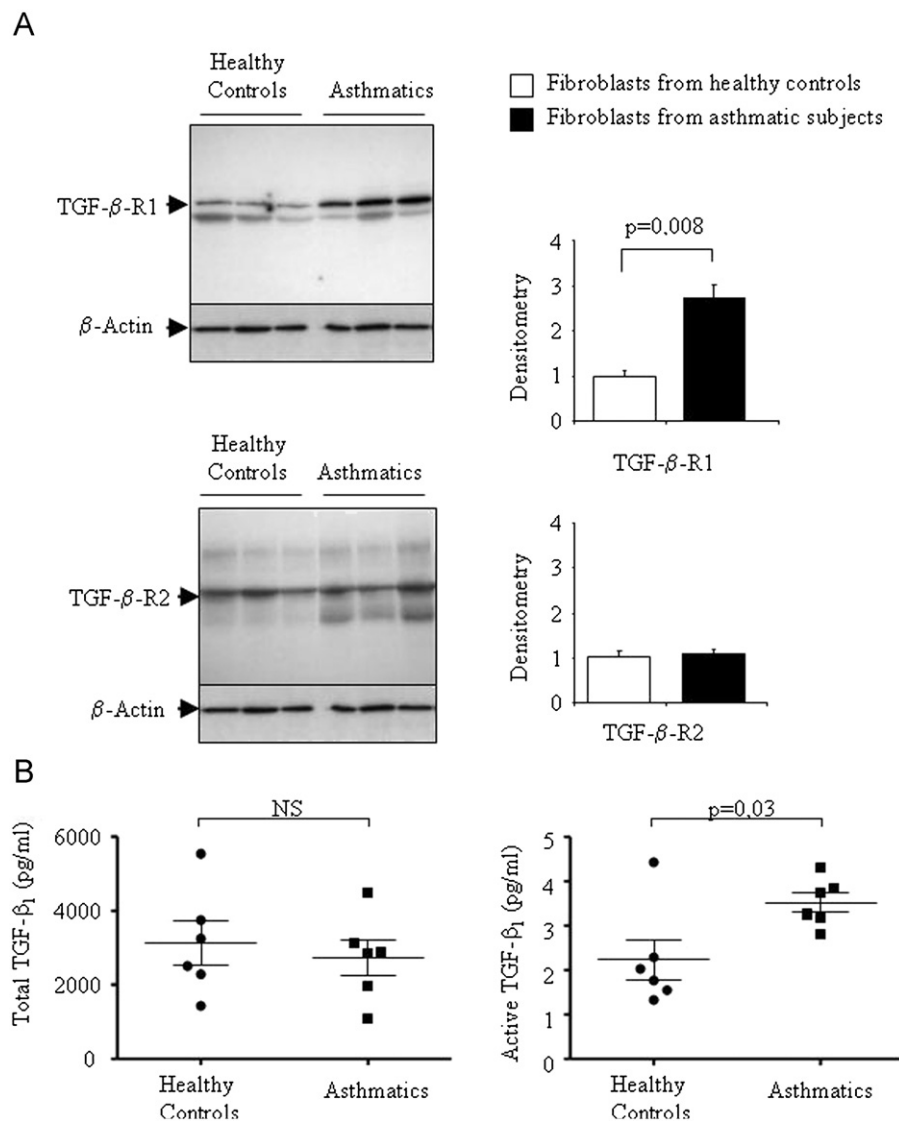


Fig. 3. TGF- β receptors expression and TGF- β 1 production in bronchial fibroblasts. (A) Western blot analysis of TGF- β -R1 and TGF- β -R2 expressions in three different normal and three different asthmatic cells. Blots were normalized with β -actin and relative intensities of each sample were analyzed by densitometry. Results are represented as arbitrary units of TGF- β -R/ β -actin ratios. (B) Baseline production of total and active TGF- β 1 by bronchial normal and asthmatic fibroblasts. TGF- β 1 concentrations were normalized to cell number.

protein expression in bronchial fibroblasts from asthmatic subjects compared to cells from healthy subjects. Inhibition of TGF- β 1 receptor signaling in fibroblasts from asthmatics blocked LTD₄-induced procollagen I(α 1) expression suggesting that the effect of LTD₄ is dependent on TGF- β 1.

CysLTs have been shown to be potent bronchoconstrictors and important inflammatory mediators for asthma. It has been suggested from animal models of asthma that CysLTs participate in airway remodeling [19,26]. In a pilot study, CysLTs measured in exhaled breath condensate from children was correlated with reticular basement membrane thickening [27]. Henderson et al. showed that treatment of mice model of asthma that had developed an established airway remodeling with CysLTs receptor antagonist reversed smooth muscle cell layer thickening and sub-epithelial fibrosis [28]. Montelukast reduced proliferation and α -actin expression by myofibroblasts from interstitial lung diseases [29]. We reported that 8 weeks of treatment with montelukast significantly reduced the number of myofibroblasts present in the airways of asthmatic subjects following low dose allergen challenge compared to baseline values [30]. This suggests that CysLTs

may play a role in the accumulation of myofibroblasts underneath the basement membrane.

CysLTs act through their specific receptors CysLT1R and CysLT2R. Immunohistochemistry combined to in situ hybridization have shown that CysLT1R is highly expressed in bronchial mucosa of asthmatic subjects compared to non-asthmatics. This receptor was localized to inflammatory cells except T cells [31]. In normal lung tissue, CysLT1R was localized to macrophages and smooth muscle [10]. CysLT1R expression was increased in nasal mucosal inflammatory cells in aspirin-sensitive patients while CysLT2R was not up-regulated. We showed an elevated expression of CysLT1R by bronchial fibroblasts from asthmatics compared to cells from non-asthmatics. Yoshisue et al. have reported that primary bronchial human fibroblasts from healthy controls express a weak signal for CysLT1R [32]. This observation is challenged by other studies showing that these cells express this receptor, express constitutively 5-lipoxygenase and have the enzymatic machinery to produce CysLTs [33]. Recently, Tufvesson et al. showed that expression of CysLT1R was higher in fibroblasts from peripheral airways compared to fibroblasts from controls [34].

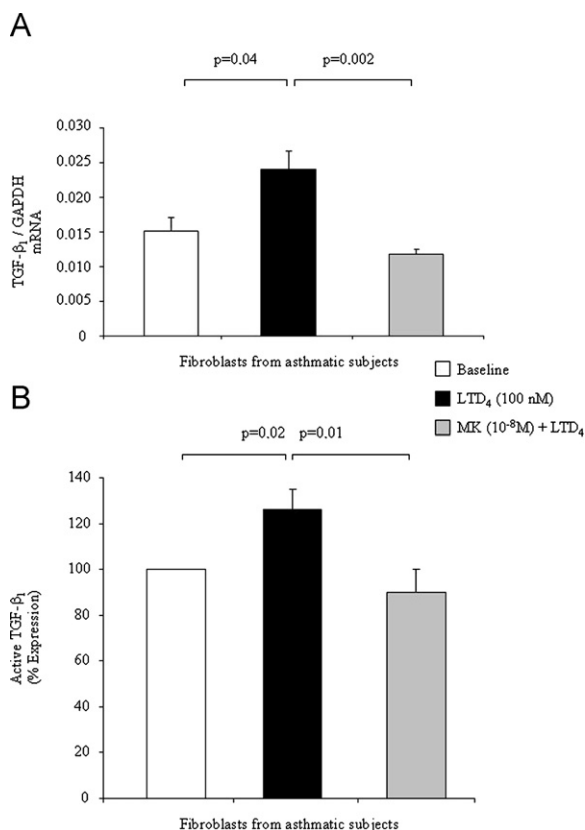


Fig. 4. Procollagen I(α₁) modulation by CysLTs in bronchial fibroblasts. (A) Real-time PCR analysis of procollagen I(α₁) mRNA after stimulation with LTD₄ (100 nM). Results are presented as collagen 1 (α₁)/GAPDH ratios. (B) Real-time PCR analysis of procollagen I(α₁) mRNA after stimulation with LTD₄ (100 nM) and/or MK-0476 (MK; 10⁻⁶ M and 10⁻⁸ M). Results are presented as collagen 1 (α₁)/GAPDH ratios. (C) N-terminal peptide of type I procollagen (PINP) production by bronchial normal fibroblasts or bronchial asthmatic fibroblasts stimulated with LTD₄ and/or MK-0476 (MK; 10⁻⁸ M). Data were obtained from 8 controls and 8 asthmatics and PINP production was normalized to cell number.

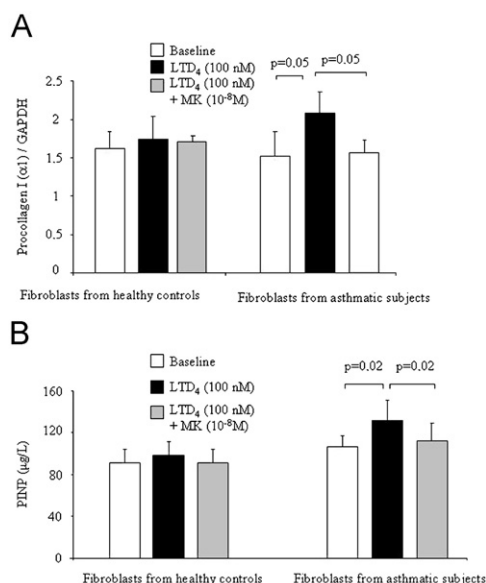


Fig. 5. TGF-β₁ modulation by LTD₄ in bronchial fibroblasts from asthmatics. (A) Real-time PCR analysis of TGF-β₁ mRNA after two hours stimulation with LTD₄ (100 nM) and/or MK-0476 (MK; 10⁻⁸ M). (B) Active TGF-β₁ two days after stimulation with LTD₄ (100 nM) and/or MK-0476 (MK; 10⁻⁸ M). Active TGF-β₁ concentrations were normalized to cell number and expressed as percentage expression of corresponding baseline.

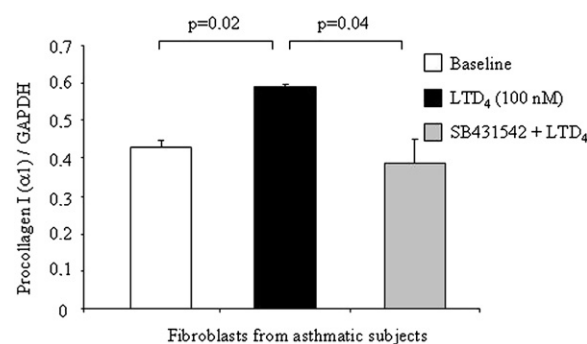


Fig. 6. TGF-β₁ implication in LTD₄-induced collagen production. Real-time PCR analysis of procollagen I(α₁) two hours after stimulation with LTD₄ (100 nM) pretreated or not with SB431542 10 μM, a TGF-β inhibitor. Results are presented as collagen 1 (α₁)/GAPDH ratios.

CysLTs induces procollagen I(α₁) mRNA and protein expression in bronchial fibroblasts from asthmatic donors. This effect was observed quickly, suggesting a possibly direct effect of LTD₄, a CysLTs that have a strong affinity for CysLT1R. Antagonist MK-0476 inhibited LTD₄-induced collagen expression, indicating that collagen modulation goes through this receptor. Targeted disruption of CysLT1R in bleomycin-induced pulmonary fibrosis in mice has been shown to aggravate pulmonary inflammation and fibrosis [35].

CysLT1R receptor upregulation by TGF-β₁ has been associated with bronchial smooth muscle cell proliferation in response to LTD₄ [16]. We have found that CysLTs can directly affect TGF-β₁ by inducing quick mRNA expression and protein activation. It has already been shown that CysLTs can synergize with TGF-β₁ to induce collagen expression [20]. The authors did not find any effect of LTD₄ alone on fetal lung myofibroblasts. To our knowledge, our study is the first to show a direct effect of CysLTs on collagen expression and TGF-β₁ production. Combined with increased TGF-β₁ receptor expression in asthmatic fibroblasts, our findings suggest that LTD₄ activates TGF-β₁ production which in turn acts on collagen mRNA and protein expression.

Our findings indicate that bronchial fibroblasts obtained from asthmatic airways express high level of CysLT1R suggesting that they are able to respond to leukotriene stimulation. MK-0476, a CysLTs receptor antagonist, inhibit LTD₄-induced procollagen I(α₁) mRNA and protein expression by these cells. This suggests that this molecule may play a role at least in part in regulating collagen deposition in asthma.

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