

Sputum Levels of Transforming Growth Factor- β_1 in Asthma: Relation to Clinical and Computed Tomography Findings

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■ Abstract

Background: Transforming growth factor (TGF) β_1 is considered to play central roles in the pathogenesis of airway remodeling in asthma. This notion is based primarily on the results of experimental studies; clinical evidence is limited.

Objectives: To ascertain the involvement of TGF- β_1 in asthma.

Methods: We studied 27 patients with moderate-to-severe, but stable, asthma treated with inhaled corticosteroids and 8 healthy controls. Helical computed tomography scans were acquired at full inspiration. Airway wall thickness (WT) was assessed on the basis of wall area corrected for body surface area (WA/BSA) and absolute WT corrected for BSA (WT/ \sqrt{BSA}) according to a validated method. Induced sputum concentrations of TGF- β_1 were measured by enzyme-linked immunosorbent assay. Pulmonary function was evaluated.

Results: Indices of expiratory airflow were significantly lower in the asthmatic patients than in the controls. WA/BSA, WT/ \sqrt{BSA} , and sputum concentrations of TGF- β_1 were significantly higher in the asthmatic patients. Sputum TGF- β_1 concentrations correlated positively with WA/BSA and WT/ \sqrt{BSA} and negatively with forced expiratory volume in 1 second in both asthmatic and control subjects.

Conclusions: Levels of TGF- β_1 in induced sputum are elevated in asthmatic patients despite treatment with inhaled corticosteroids and are associated with airflow obstruction and airway wall thickening. TGF- β_1 is involved in the pathogenesis of airway remodeling and resultant functional impairment and it may be a target for specific medical treatment.

Key words: Asthma. Transforming growth factor β_1 . TGF- β_1 . Airway remodeling. Computed tomography. Induced sputum.

■ Resumen

Antecedentes: Se considera que el factor del crecimiento transformante (FCT) β_1 tiene un papel central en la patogenia del proceso de remodelado de las vías respiratorias en el asma. Esta idea está basada, ante todo, en los resultados de estudios experimentales y los datos clínicos son limitados.

Objetivo: Averiguar qué implicación tiene el FCT- β_1 en el asma.

Métodos: Se estudiaron 27 pacientes que padecían asma de moderada a grave, pero estable, tratada con corticoesteroides inhalados y 8 controles. Se obtuvo una tomografía computerizada helicoidal realizada en inspiración profunda. El grosor de las paredes (GP) de las vías respiratorias se evaluó en base al área de pared corregida por área de superficie corporal (AP/ASC) y el GP absoluto corregido por ASC (GP/ \sqrt{ASC}) según un método validado. Las concentraciones de esputo inducidas de FCT- β_1 se midieron mediante ensayo inmunoenzimático de adsorción (ELISA). También se evaluó la función respiratoria.

Resultados: Los índices de flujo espiratorio fueron significativamente más bajos en los pacientes con asma que en los controles. Los resultados de AP/ASC y GP/ \sqrt{ASC} y las concentraciones de esputo del FCT- β_1 fueron significativamente más elevadas en los pacientes asmáticos que en los controles. Las concentraciones de esputo del FCT- β_1 se correlacionaron positivamente con el AP/ASC y GP/ \sqrt{ASC} , y negativamente con el volumen espiratorio forzado en el primer segundo, tanto en los sujetos asmáticos como en los controles.

Conclusiones: Las concentraciones de FCT- β_1 en el esputo inducido son elevadas en los pacientes asmáticos a pesar del tratamiento con corticoesteroides inhalados y se asocian con la obstrucción del flujo de aire y el aumento del grosor de las paredes de las vías respiratorias. El FCT- β_1 está implicado en la patogenia del proceso de remodelado de las vías respiratorias y en la insuficiencia funcional resultante y puede ser un objetivo para el tratamiento médico específico.

Palabras clave: Asma. FCT- β_1 . Proceso de remodelado de las vías respiratorias. Tomografía computerizada. Inducción de esputo.

Introduction

Asthma is characterized by chronic inflammation and associated remodeling of the airways [1]. Many asthmatic patients have persistently reduced expiratory airflow indices, despite treatment with adequate doses of anti-inflammatory agents and lack of a smoking history. Such chronic or irreversible airflow limitation is now attributed to airway remodeling [1]. The pathogenesis of asthmatic airway remodeling involves goblet cell and submucosal gland hyperplasia, vascular proliferation, deposition of connective tissue in the subepithelium and submucosa, and hypertrophy and hyperplasia of airway smooth muscle, changes that may result in thickening of the whole airway wall [1].

Airway wall remodeling may result from an imbalance between tissue regeneration and repair mechanisms. The airway epithelium may undergo repeated episodes of injury and repair in asthma, associated with high expression of growth factors such as transforming growth factor (TGF) β and epidermal growth factor. TGF- β belongs to a family of growth modulating cytokines, existing in three isoforms (β_1 , β_2 , and β_3) [2]. TGF- β_1 stimulates proliferation of fibroblasts and influences the turnover of matrix proteins. This isoform is considered to play a central role in the pathogenesis of airway remodeling, participating in processes such as subepithelial fibrosis and smooth muscle enlargement [2-4]. However, its precise roles in asthma remain to be fully elucidated *in vivo*.

Computed tomography (CT) has recently been used to indirectly yet noninvasively assess airway remodeling [5-10]. CT can assess a broader range of airway and lung dimensions than other techniques, although histologic details remain unclear. In this study, we evaluated sputum concentrations of TGF- β_1 , pulmonary function, and the thickness of airway walls as assessed by helical CT in asthmatic and normal subjects to analyze correlations among these variables.

Materials and Methods

Subjects

We studied 27 patients with stable asthma who were regularly visiting the outpatient asthma and cough clinic of Kyoto University Hospital. Asthma was diagnosed according to criteria of the American Thoracic Society [11]. All patients were considered to have stable asthma because the disease had been fully controlled for at least 1 month. All patients received inhaled corticosteroids daily, at a dose equivalent to a mean (SD) of 800 (248) μ g of chlorofluorocarbon beclomethasone, and short-acting inhaled β_2 -agonists on demand. The clinical severity of asthma according to the criteria of the Global Initiative for Asthma [12] was step 3 (moderate persistent) in 22 patients and step 4 (severe persistent) in 5. Eight healthy controls with no history of respiratory disease were also studied.

No subject had ever smoked cigarettes. CT scans, pulmonary function tests, and induced sputum tests were performed in that order within a period of 2 weeks. The study

was approved by the ethics committee at our institution, and written informed consent was obtained from all subjects.

CT Scans and Analysis

CT scanning and analysis of airway wall dimensions were performed as described previously [6-8,13,14]. Briefly, helical CT scanning was performed at 120 kV, 50 mA, 3 mm collimation, using a scan time of 1.0 second with a Toshiba X-Vigor CT scanner (Toshiba, Tokyo, Japan). Scans were acquired at full inspiration, and images were reconstructed using the FC10 algorithm at 2-mm intervals. Images including the trunk of the apical bronchus of the right upper lobe were selected by a consensus reading between two pulmonologists (M.Y. and A.N.). Airway wall area (WA) and absolute wall thickness (WT) were measured automatically on a personal computer according to our validated method [14]. WA and WT were corrected for body surface area (BSA) and the square root of BSA [6-8,13,14].

Sputum Induction and Processing

Sputum induction and processing were performed as previously described [9]. In brief, the subjects were premedicated with inhaled salbutamol (200 μ g) and then inhaled hypertonic (3%) saline solution, administered by an ultrasonic nebulizer (MU-32, Azwell Inc, Osaka, Japan) for 15 minutes.

All adequate plugs of sputum were separated from saliva and weighed. The plugs were treated with 0.1% dithiothreitol (Squatasol, Oxoid Ltd, Hampshire, UK), followed by treatment with the same volume of Dulbecco's phosphate-buffered saline solution (PBS). After centrifugation, sputum supernatants were stored at -80°C , and cells were stained by the May-Grünwald-Giemsa method. Cell differential counts were determined by counting at least 400 nonsquamous cells.

Measurement of TGF- β_1 in Sputum Supernatants

Sputum induction and processing were performed as previously described [9]. Induced sputum concentrations of TGF- β_1 were examined by enzyme-linked immunosorbent assay with a use of a commercially available kit according to the manufacturer's instructions (Promega Corporation, Madison, Wisconsin, USA). All samples underwent acid pretreatment as described previously [15].

Pulmonary Function

Pre-bronchodilator values of forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC , and forced mid-expiratory flow ($\text{FEF}_{25\%-75\%}$) were measured with a Chestac-65V unit (Chest MI Corp, Tokyo, Japan).

Statistical Analysis

Values are presented as means (SD). Statistical analysis included use of the Mann-Whitney U test and calculation of the Spearman rank correlation coefficient (ρ) (Stat View software, SAS Institute Inc, Cary, North Carolina, USA). Statistical significance was defined as $P < .05$.

Results

The characteristics of the asthmatic and healthy subjects are shown in the Table. Age, sex distribution, and body surface area significantly differed between the two groups. The asthmatic patients had more airway obstruction than the controls, as demonstrated by significantly lower FEV_1/FVC and $FEF_{25\%-75\%}$, but not FEV_1 . The values of WA/BSA and WT/\sqrt{BSA} of the patients were greater than those of the controls, consistent with the findings of previous studies [6,8].

Sputum $TGF-\beta_1$ concentrations in the asthmatic patients were more than double the control values and the difference was statistically significant (Table). Sputum levels of $TGF-\beta_1$ marginally correlated with the number of eosinophils ($\rho = 0.29$, $P = 0.09$), but not with the number of macrophages or neutrophils ($\rho = -0.02$ and $\rho = 0.02$ respectively; $P = 0.92$ for both) in the asthmatic and control subjects analyzed together.

Sputum concentrations of $TGF-\beta_1$ positively correlated with both WA/BSA and WT/\sqrt{BSA} (Figure 1) and negatively correlated with FEV_1 (Figure 2).

Table. Characteristics of Asthmatic Patients and Healthy Controls

	Asthma (n=27)	Control (n=8)	P
Age, y	62.1 (9.8)	33.1 (4.8)	<.0001
Male/female	7/206/2	.012	
Body surface, m ²	1.56 (0.18)	1.81 (0.22)	.011
FEV_1 , % predicted	93 (24)	101 (11)	.44
FEV_1/FVC , %	71 (11)	82 (8)	.015
$FEF_{25\%-75\%}$, % predicted	69 (34)	98 (29)	.04
WT/\sqrt{BSA} , mm/m	.4 (0.2)	1.1 (0.1)	.0002
WA/BSA , mm ² /m ²	25.4 (4.4)	17.1 (1.8)	.0001
Sputum indices			
Eosinophils, %	11.1 (17.4)	0.8 (1.4)	.002
Neutrophils, %	56.9 (21.8)	70.9 (13.2)	.15
Macrophages, %	32.0 (7.3)	28.1 (12.0)	.72
$TGF-\beta_1$ levels, pg/mL	2759 (1685)	1249 (843)	.011

Abbreviations: BSA, body surface area; $FEF_{25\%-75\%}$, forced midexpiratory flow; FEV_1 , forced expiratory volume in 1 second; FVC, forced vital capacity; TGF, transforming growth factor; WA, wall area; WT, wall thickness.

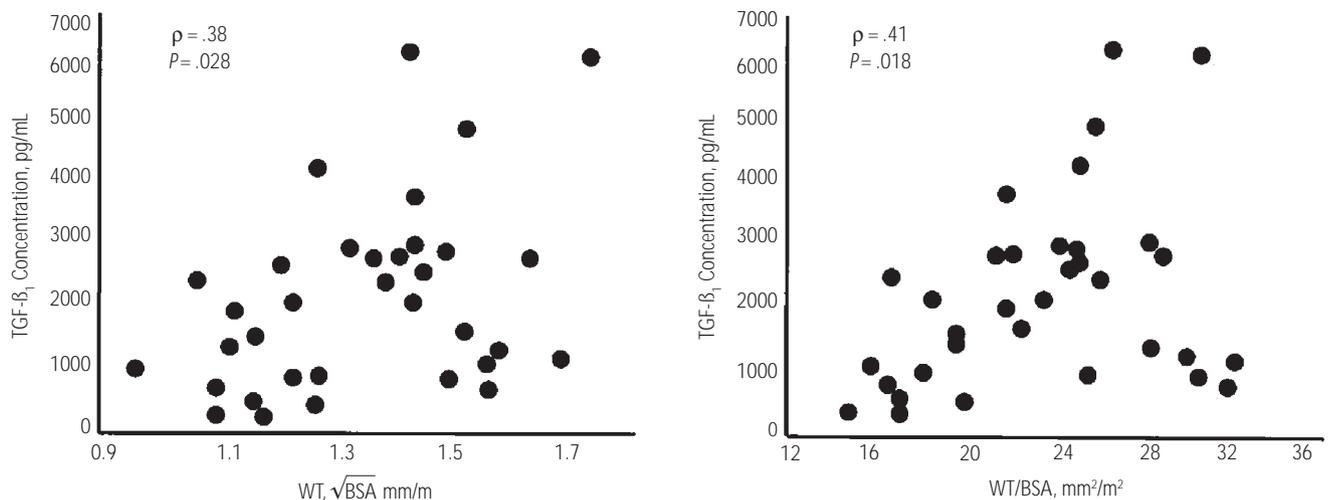


Figure 1. Correlation (Spearman coefficient, ρ) between indices or airway wall thickness and transforming growth factor ($TGF-\beta_1$) concentrations in induced sputum. BSA indicates body surface area; WA, airway wall area.

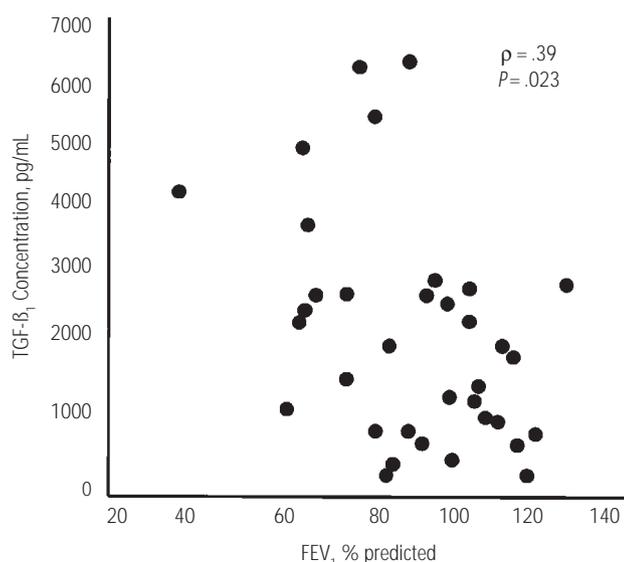


Figure 2. Correlation (Spearman coefficient, ρ) between forced expiratory volume in 1 second (FEV_1) and induced sputum concentration of transforming growth factor (TGF) β_1 .

Discussion

Our study showed that patients with moderate-to-severe asthma treated with inhaled corticosteroids had elevated TGF- β_1 levels in sputum, associated with airway wall thickening and airflow limitation. To our knowledge, this is the first study to explore the relation between levels of airway TGF- β_1 and airway WT as assessed by CT.

In asthmatic airways, TGF- β is most likely derived from different types of inflammatory or structural cells, including eosinophils, neutrophils, alveolar macrophages, fibroblasts, and epithelial cells [2-4]. In asthmatic patients, Vignola et al [3] demonstrated significantly higher epithelial or submucosal cell expression of TGF- β . Furthermore, the higher expression correlated with basement membrane thickness and mucosal fibroblast number. Minshall et al [4] also found elevated subepithelial cell expression of TGF- β_1 mRNA; this variable correlated with the degree of subepithelial fibrosis and the number of activated eosinophils (EG2-positive). In our analysis of sputum, there was a tendency for TGF- β_1 levels to correlate positively with eosinophil count, consistent with previous observations [2-4].

Previous studies have shown that airway walls are thicker in asthmatic subjects than in health controls, as assessed by CT, and that this variable correlates with functional indices such as severity of disease and airflow obstruction [5-8]. More recently, associations between the thickness of airway walls and biomarkers of airway inflammation and remodeling have been investigated. Thickness correlates positively with sputum levels of tissue inhibitor of metalloproteinases-1 correlates negatively with the molar ratio of matrix metalloproteinase-9 to tissue inhibitor of metalloproteinases-1 [14]. It also correlates

with eosinophilic cationic protein levels in bronchoalveolar lavage in asthmatic children [10]. Our study indicates that TGF- β_1 may also contribute to airway wall thickening.

Our asthmatic patients were treated with sufficient doses of inhaled corticosteroids to control symptoms, but they still had significantly higher sputum TGF- β_1 levels than the controls. Elevated levels of TGF- β_1 in sputum may be attributable to uncontrolled airway inflammation, as suggested by the higher sputum eosinophil count in patients than in controls. Since corticosteroid treatment might not attenuate TGF- β expression or collagen deposition in the airway mucosa [16], the prevention or reversal of airway remodeling may require specific treatment targeting TGF- β , as demonstrated experimentally in rats [17].

The differences in age and sex distribution between the asthmatic and control subjects may be a limitation of our study. However, to our knowledge, there is no evidence that TGF- β production or metabolism might be different relative to age or sex. Indeed, no such association was observed in our asthmatic or healthy subjects (data not shown). Moreover, in our previous study, TGF- β serum levels showed no correlation with age or sex in stable asthmatic patients [18].

In conclusion, the protein levels of TGF- β_1 in induced sputum are elevated and associated with airflow limitation and airway wall thickening in patients with moderate-to-severe asthma treated with inhaled corticosteroids. These results indicate the involvement of TGF- β_1 in the pathogenesis of airway remodeling in asthmatic patients and suggest the need for specific medical treatment targeting this factor.

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