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Elevated levels of transforming growth factor-β_1 in serum of patients with stable bronchiectasis

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Summary Bronchiectasis is a chronic inflammatory and infective airway disease characterized by irreversible dilatation of the bronchi and persistent purulent sputum. Transforming growth factor-β_1 (TGF-β_1) has been found to be increased in the lungs or bronchoalveolar lavage fluid of patients with inflammatory lung diseases. However, little is known on the serum TGF-β_1 levels in patients with bronchiectasis. We aimed to determine the serum TGF-β_1 concentrations in 95 patients with stable bronchiectasis (63 women; mean $\pm$ SD age, 58.9 $\pm$ 14.1 years) and 68 control subjects (23 women; 48.9 $\pm$ 12.8 years) by ELISA, and to correlate with clinical parameters. The serum TGF-β_1 levels were significantly higher in bronchiectatic patients compared with control subjects (median [range], 1812.5 pg/ml [1226.4–4114.5 pg/ml] vs. 1342.4 pg/ml [940.3–2371.7 pg/ml]; $P<0.001$). There was, however, no correlation between serum TGF-β_1 levels with FEV_1 (% predicted), FVC (% predicted), 24 h sputum volume, the number of bronchiectatic lung lobes or total white blood cell count ($P>0.05$). Our findings support previous indications that TGF-β_1 may contribute to bronchiectatic airway inflammation. Further studies on the potential mechanisms and pathogenesis implications of this elevation should also be pursued in future.

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Introduction

Bronchiectasis is a chronic infective and inflammatory airway disease with diverse aetiology that is very common among the Chinese, and is characterized by irreversible dilation of the bronchi and persistent purulent sputum production. High levels of proinflammatory cytokines are present in airway secretions, and neutrophils are the predominate cells in the airway lumen.[1] In patients with bronchiectasis, bronchial damage is thought to exist due to neutrophil inflammatory products released in response to bacterial infection.[2] Chronic colonization with *Pseudomonas aeruginosa* (PA) is associated with extensive lung disease and severe airflow obstruction.[3]

The mechanisms underlying airway inflammation in bronchiectasis are unknown. In other chronic inflammatory diseases, the generation and release of potent growth and activating factors for fibroblasts is particularly important. Transforming growth factor β (TGF-β) has emerged as one of the mediators, which have been implicated in repair following lung injury. The mammalian TGF-β family comprises three main isoforms designated as TGF-β_1, TGF-β_2, and TGF-β_3.[4] TGF-β_1 is one of the most profibrotic growth factors and has been implicated in formation of extracellular matrix in pulmonary fibrosis.[5] Animal studies have shown that TGF-β_1 has an inhibitory effect on immunoglobulin synthesis by lymphocytes and can suppress airway inflammation.[6,7] TGF-β_1 levels have been reported to be either an increase or no change in bronchoalveolar lavage (BAL) fluid, bronchial biopsy specimens and plasma from patients with fibrotic lung disease, asthma and chronic obstructive pulmonary disease (COPD).[8–13] To our knowledge, however, there have been no previous reports regarding serum levels of TGF-β_1 in stable bronchiectasis. Clinicopathologic correlation of systemic TGF-β_1 is also unknown in patients with bronchiectasis. We have, therefore, performed this study to evaluate the serum level of TGF-β_1 in a cohort of 95 patients with clinically stable bronchiectasis in comparison to 68 healthy control subjects, and to correlate with clinical parameters.

Materials and methods

Subject recruitment

Consecutive patients with proven bronchiectasis who were not treated with inhaled steroid therapy and diagnosed by high-resolution computed tomography (HRCT) were recruited from the specialist respiratory clinics of the University of Hong Kong with written informed consent (participation rate 95%). Inclusion criteria included absence of asthma, COPD, tuberculosis, or other unstable systemic diseases; no alteration in medication and dosage for at least 3 months; and steady-stable bronchiectasis. The latter was defined as the absence of significant (i.e. $<$20%) alteration of 24 h sputum volume, forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC), or changes in respiratory symptoms for three consecutive weeks.[14] All patients were clinically known as idiopathic bronchiectasis, which is the most common in Hong Kong. Out of all patients, there were 14 patients with antibiotic treatment (1 with clarithromycin, 5 with levofloxacin, 5 with erythromycin, 2 with gentamycin and 1 with streptomycin). Healthy control subjects who were receiving no regular medications and were asymtomatic of respiratory, cardiovascular, GI, renal, and neurologic diseases were also recruited randomly from the community with written informed consent (participation rate 45%). This study had the approval from the Ethics Committee, Faculty of Medicine, The University of Hong Kong.

Parameters assessed in patients with bronchiectasis

The bronchiectatic patients were questioned about the presence of respiratory symptoms, including cough, dyspnea, haemoptysis, sputum production, chest pain, and wheezing, and they were examined physically. The number of lung lobes (including lingual as an individual lobe) affected by bronchiectasis was determined by a thoracic radiologist who examined the HRCT scan of each patient using standard criteria.[15] Briefly, bronchiectais was present when the bronchial segment or sub-segment appeared larger than the accompanying artery on HRCT. The volume of a 24-h sputum production was also determined for each patient as the mean of three consecutive 24-h collections performed at steady state. Spirometry (FEV_1 and FVC), expressed as percent predicted, was measured with a SensorMedics 2200 (Sensor-Medics, Yorba Linda, USA) package.

Blood collection and storage

Clot blood is taken from each subject. Serum was separated at 1600 $\times$ *g* for 10 min (4 °C) and stored at -70 °C until assayed for TGF-β_1.

Determination of serum TGF-β_1 levels

Levels of TGF-β_1 in serum were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (BD OptEIA™ Set for human TGF-β_1; San Diego, CA, USA) according to the manufacturer's instructions. Serum samples were activated before assay by acidification using 1 M HCl, and diluted with assay buffer bringing TGF-β_1 values of all the diluted samples within the linear range of the standard curve (62.5–4000 pg/ml). The limit of detection of the assay was 62.5 pg/ml. All measurements were performed in duplicate. All samples from patients and controls were measured in adjacent wells to minimize assay variability. The reproducibility, calculated as the coefficient of variation (CV) of intra- and interassay variability, was 3.5% and 7.5%, respectively.

Microbiological assessment of sputum

Fresh sputum was obtained for microbiological evaluation.[16] Standard microbiological procedures were employed to identify all the sputum bacteria and classify them into PA and others.

Statistical analysis

Data were shown as mean $\pm$ SD or median (range). Preliminary inspection of data revealed that serum TGF-β_1 levels were not normally distributed. Comparisons between groups were made using the non-parametric Mann–Whitney rank order test. Correlations were evaluated by Spearman's rank method. A P value of <0.05 was taken as statistically significant. The analysis was performed using the statistical software (SPSS version 11.0; SPSS; Chicago, IL, USA).

Results

Control subjects and patients

Table 1 shows subject demography and clinical features. There was significant difference between age ($P<0.001$) and gender ($P<0.001$) in the two groups. Patients with bronchiectasis were significantly older, and more women than control subjects. Of the patients with bronchiectasis, 19 patients were either ex- or current smokers, 26 and 10 patients had colonization with potential pathogenic microorganisms such as PA or *Haemophilus influenza* (HI), respectively, in their sputum. There was only one patient colonized with *Mycobacterium chelonae* in the sputum. Other patients had found colonization with non-potential pathogenic microorganisms such as commensals in their sputum. Twenty-five control subjects were also either ex- or current smokers in this study.

Serum TGF-β_1 levels

Patients with bronchiectasis showed a significantly higher level of serum TGF-β_1 than control subjects (median [range], 1812.5 pg/ml [1226.4–4144.5 pg/ml] vs. 1342.4 pg/ml [940.3–2371.7 pg/ml]; $P<0.001$) (Fig. 1). There was no significant difference in serum TGF-β_1 levels among nonsmokers and smokers in either control subjects (1163.5 pg/ml [993.3–2177.9 pg/ml] vs. 1654.3 pg/ml [905.6–2414.8 pg/ml] for nonsmokers and smokers, respectively) or patients with stable bronchiectasis (1845.5 pg/ml [1241.0–3977.4 pg/ml] vs. 1446.5 pg/ml and [1196.3–4525.7 pg/ml] for nonsmokers and smokers, respectively). There was also no significant difference in serum TGF-β_1 levels between stable bronchiectais patients with PA or HI

Table 1 Characteristics of study subjects.

	Healthy controls	Patients with bronchiectasis
N	68	95
Male:female	45:23	32:63***
Age (yrs)*	48.9 $\pm$ 12.8	58.9 $\pm$ 14.1***
Smokers	25	19
FEV_1% predicted*	Not done	74.3 $\pm$ 29.1
FVC % predicted*	Not done	84.0 $\pm$ 23.0
FEV_1/FVC*	Not done	67.5 $\pm$ 15.5
Median 24-h sputum volume (range, ml)	N/A	10 (5–25)
No. of bronchiectatic segments*	N/A	3.0 $\pm$ 1.6
Median total white blood cell count ($\times 10^6$/ml)	N/A	6.5 (4.9–8.0)

***$P<0.001$ obtained by comparing data between healthy controls and patients with stable bronchiectasis.
*Data are presented as mean $\pm$ SD unless otherwise indicated.

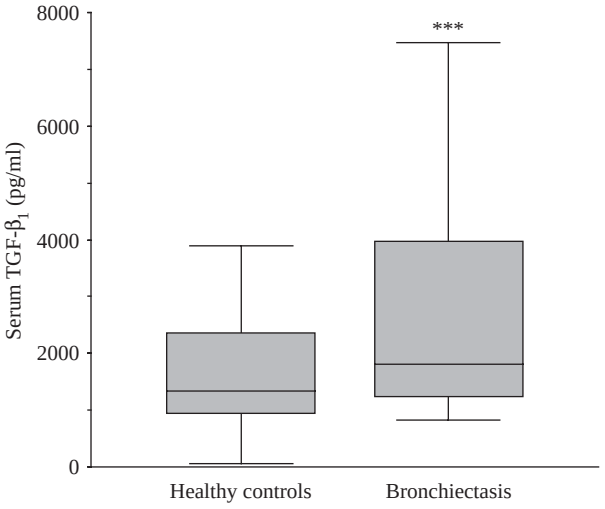

Figure 1 Serum TGF-β_1 levels in healthy controls ($n = 68$) and patients with stable bronchiectasis ($n = 94$). Median (interquartile range) is shown. ***$P < 0.001$.

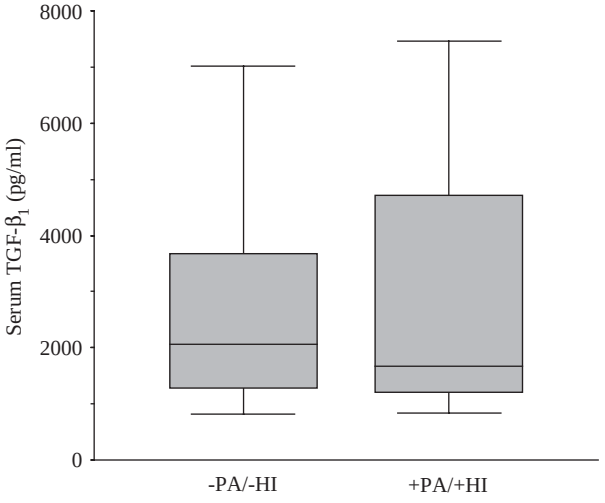

Figure 2 Serum TGF-β_1 levels in stable bronchiectatic patients without *P. aeruginosa* (- PA) or *H. influenzae* (−HI) colonization ($n = 58$) and with PA (+PA) or HI (+HI) colonization ($n = 36$). Median (interquartile range) is shown. No significant difference between the two groups was found.

colonization (1672.0 pg/ml [1200.7–4780.5 pg/ml]) compared with those without PA or HI colonization (2069.3 pg/ml [1261.8–3750.6 pg/ml]) (Fig. 2). Furthermore, patients with or without taking antibiotics showed similar levels of serum TGF-β_1 levels (1965.8 pg/ml [1192.6–3996.3 pg/ml] for patients with antibiotics [$n = 14$] vs. 1765.8 pg/ml [1230.5–4525.7 pg/ml] for patients without antibiotics [$n = 79$]; $P > 0.05$).

Table 2 Relationship between serum TGF-β_1 with clinical parameters in patients with stable bronchiectasis.

Variables	Serum TGF-β_1 (pg/ml)
24-h sputum volume, *r* (*P* value)	−0.02 (0.87)
No. of bronchiectatic segments, *r* (*P* value)	−0.12 (0.27)
FEV_1% predicted, *r* (*P* value)	−0.07 (0.52)
FVC % predicted, *r* (*P* value)	−0.06 (0.61)
FEV_1/FVC, *r* (*P* value)	0.08 (0.43)
Total white blood cell count, *r* (*P* value)	0.139 (0.20)

Relationship between TGF-β_1 level and clinically important parameters

Table 2 depicts the correlations between serum TGF-β_1 level with clinical parameters in patients with stable bronchiectasis. There was no correlation between serum TGF-β_1 level with FEV_1 (% predicted), FVC (% predicted), 24-h sputum volume, and the number of bronchiectatic lung lobes ($P > 0.05$). Correlation analysis between serum TGF-β_1 level and sputum viscosity, sputum purulence, white blood cell count or last exacerbation frequency also found no significant relationship ($P > 0.05$). However, correlation analysis showed that 24-h sputum volume correlated positively with number of lung segments affected by bronchiectasis ($r = 0.23, P < 0.05$), and negatively with FEV_1 or FVC ($r = -0.25, P < 0.05$ or $r = -0.31, P < 0.01$, respectively).

Discussion

In this study, we found that the serum TGF-β_1 level was significantly higher in Chinese patients with bronchiectasis than in control subjects after adjusting for differences in age and gender between groups as the controls were younger and less women compared with the patients. Moreover, no difference in serum TGF-β_1 level was detected among nonsmokers and smokers in either control subjects or patients with bronchiectasis. We also found no difference in serum TGF-β_1 levels between bronchiectais patients colonized with PA or HI compared with those without PA or HI colonization. Correlation analysis showed that serum TGF-β_1 level did not correlate with clinical parameters such as FEV_1, FVC, 24-h sputum

volume, or the number of bronchiectatic lung lobes.

Bronchiectasis is a very common and largely idiopathic disease among the Chinese.[17] Similar to cystic fibrosis, there are prominent chronic inflammatory and infective elements in the pathogenesis of bronchiectasis. Although our group has shown that sputum elastase levels correlate with sputum production, proinflammatory cytokine expression, and spirometry in patients with stable bronchiectasis,[18] which is consistent with previous report in BAL,[19] objective and convenient markers for the assessment of disease activity in bronchiectasis are still lacking. The potential involvement of TGF-β_1 in the airways of asthmatics and subjects with COPD has been hypothesized by several reports. However, little is known about the circulating TGF-β_1 concentration in bronchiectasis. Because growth factors are able to modulate the proliferation of fibroblasts and the synthesis of extracellular matrix components,[20] we put forward the hypothesis that TGF-β_1 may play a role in the pathogenesis of subepithelial fibrosis associated with chronic airway inflammation in bronchiectasis.

The source of elevated serum TGF-β_1 levels in our bronchiectatic patients has not been assessed in this study. Chu and co-workers have suggested that neutrophils isolated from peripheral blood showed a significant increase in expression of TGF-β_1 mRNA and protein in asthmatic patients compared with healthy controls.[21] If this is so, one might anticipate a close association between TGF-β_1 levels and the neutrophil count. In bronchiectasis, there are reports indicating that significantly higher neutorphils are present in the lamina propria of the airways, and in induced sputum.[22,23] TGF-β_1 has been documented to both recruit and activate neutrophils and to prolong neutrophil survival.[24] Therefore, the bronchial and peripheral induced sputum neutrophilia documented in bronchiectasis may associate with elevated serum TGF-β_1 levels in these patients. However, we found no correlation between the white blood cell count and serum TGF-β_1 level in our patient group. The limitation of our current study is that the elevated serum TGF-β_1 levels may not reflect the local concentrations in the lungs. Without any correlation between serum TGF-β_1 levels and numbers of lung lobes affected by bronchiectasis, this suggests that the systemic reflection is not related to the involved lung area. Other biological samples such as BAL, induced sputum and exhaled breath condensate, might be more appropriate in assessing the localization of TGF-β_1 within the respiratory tract, even though poor correlations between serum cytokines and BAL have been found previously.[19] In addition, there are polymorphisms in the promoter and in the coding region of the TGF-β_1 gene (C-509 T and T869C) that have been shown to be associated with increased production of TGF-β_1.[25,26] However, we have not genotyped our patients in this current study. The relationship between increased serum TGF-β_1 levels and genotypes or increased circulating neutrophils in bronchiectatic patients merits further investigation using a larger cohort.

On the other hand, TGF-β_1 has been documented to be activated by matrix metalloproteinase-9 (MMP-9),[27] and may be mediated via MMP-9 induced proteolytic cleavage of latent TGF-binding protein-1, resulting in release of TGF-β_1.[28] This mechanism therefore could be a link between elastolysis induced by MMP-9 and simultaneous production of fibrosis by activation of TGF-β_1. In support of this, our group has found overexpression of MMP-8 and -9 in the lamina propria of bronchiectatic airways.[29]

Previous reports have documented that infection of macrophages with certain viruses, parasites, and protozoa can induce TGF-β_1 production, which has a suppressive action on the host inflammatory response as well as an increased expression of TGF-β_1 following airway infection with parainfluenza virus in animal studies.[30–33] However, we found no difference in serum TGF-β_1 levels between bronchiectasis patients colonized with PA or HI compared with those without PA or HI colonization, in agreement with the finding that antibiotic treatment had no influence on the elevated serum TGF-β_1 levels in this group of clinically stable bronchiectasis. The discrepancies might be due to the microorganisms isolated in our study, in contrast to *Mycobacteria tuberculosis* or *Chlamydia trachomatis*.[33] There was only one patient colonized with *M. chelonae* found in the sputum from this study. Therefore, increased serum TGF-β_1 levels in bronchiectasis are not reflected by post-infective phenomenon that serves to down-regulate the host immune response.

In conclusion, the results of our current study clearly show that Chinese patients with stable bronchiectasis had evidence of elevated serum TGF-β_1 levels, which probably contributed to the pathogenesis of this disease. Clearly, the role of increased serum TGF-β_1 levels in bronchiectasis during stable condition and exacerbation merits further investigation.

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