Transforming growth factor β_1 gene expression in human airways

John-David Aubert, Bakul I Dalal, Tony R Bai, Clive R Roberts, Shizu Hayashi, James C Hogg

Abstract

Background **– Asthmatic airways have a characteristic deposition of connective tissue under the epithelial basement membrane, but the mediators involved in this alteration are unknown. Several authors have postulated that transforming growth factor β_1 (TGF-β_1) could be overexpressed in asthmatic airways.**

Methods **– Lung samples from 16 asthmatic patients, six patients with chronic obstructive pulmonary disease (COPD), and six non-obstructed smokers were analysed. RNA was extracted from these tissues to measure expression of TGF-β_1 by Northern blot analysis using a cDNA probe for TGF-β_1. The level of expression was quantitated by densitometry using glyceraldehyde 3-phosphate dehydrogenase mRNA as a control. TGF-β_1 was localised to specific cell types in these lungs by immunohistochemical analysis using polyclonal antibodies specific for intracellular and extracellular TGF-β_1.**

Results **– The 2·5 kb TGF-β_1 mRNA was seen in all 18 samples analysed by Northern blotting and densitometric analysis showed no difference between the asthmatic group (mean (SD) 108% (43%)), the group with COPD (122% (33%)), and the non-obstructed group (100% (49%)). The TGF-β_1 precursor was immunolocalised throughout the airway wall including the epithelium and in alveolar macrophages. The mature TGF-β_1 was localised primarily within the connective tissue of the airway wall. These patterns of expression of both forms of TGF-β_1 were similar in lungs from asthmatic patients, those with COPD, and controls.**

Conclusions **– While TGF-β_1 mRNA and protein are abundantly expressed in human lungs, there is no clear difference in expression between the airways of asthmatic subjects and those of smokers with and without COPD.**

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The airway wall from patients with asthma is thickened by an increase in the amount of several of its tissue components including smooth muscle, connective tissue, and mucous glands.[1–3] In particular, connective tissue deposition beneath the epithelial basement membrane has been described.[4] Together with type I, III, and V collagen fibres, the numbers of myofibroblasts containing α-actin are increased.[5] These findings are present even in patients with mild asthma, and seem to persist despite long term treatment with glucocorticoids.[6] Airway wall thickening may have clinical significance in asthmatic subjects as it has been shown by mathematical modelling that an increase in the inner wall area can lead to exaggerated narrowing of the airway lumen with normal smooth muscle shortening.[17] Similar but less severe structural changes have also been reported in chronic obstructive pulmonary disease (COPD) associated with cigarette smoking.[8,9] The biological factors responsible for the increased tissue mass in these diseased airways have not been identified, and the present study was designed to investigate the possible role of transforming growth factor β (TGF-β) in the structural changes of the airway wall in patients with asthma and COPD.

Transforming growth factors are a family of growth regulators that play an important part in the metabolism of the extracellular matrix.[10,11] They promote matrix protein synthesis,[12] downregulate the proteolytic enzymes involved in matrix degradation,[13] and increase the expression of receptors for extracellular matrix proteins.[14,15] Based on these properties, the potential involvement of TGF-β in the airways of asthmatic subjects has been hypothesised by several authors.[16,17] Three closely related subtypes of TGF-βs, TGF-β_1, TGF-β_2, and TGF-β_3, have been identified in mammalian species. These molecules all bind to the same heterodimeric receptor I–II on the cell surface[18–20] and, although their biological activity is qualitatively similar, their expression is regulated through distinct pathways.[21] TGF-β_1 is synthesised as a 390 amino acid latent precursor which is processed by proteolytic cleavage to a 112 amino acid molecule which, by formation of disulphide bridges, yields a biologically active homodimer. Although activation from the latent precursor into the mature peptide can be achieved in vitro by proteases such as plasmin or cathepsin D, by an acidic environment, or by chaotropic agents,[22,23] the mechanisms that control this activation in vivo are poorly understood.

As a first approach in analysing the potential role of TGF-β_1 in airway wall thickening, a survey of this growth factor in lung tissue from patients with asthma, COPD, and non-obstructed control patients was made. TGF-β_1 mRNA expression was measured by Northern blotting and the TGF-β_1 protein localised to specific cell types by immunohistochemistry in these samples. These data were then used to test the hypothesis that overexpression of

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Table 1 Clinical data

	Non-obstructed smokers (n=6)	*Smokers with COPD (n=6)*	*Asthma (surgery) (n=4)*	*Asthma (necropsy) (n=12)**
Mean (range) age (years)	63 (49–70)	69 (62–83)	62 (47–72)	34 (4–72)†
Sex (M:F)	4:2	5:1	2:2	5:7
Smoking (pack years)‡	38 (15)	77 (27)§	44 (8)¶	0
FEV_1 (% predicted)	92 (9)	55 (11)|	94 (14)**	NA
FEV_1/FVC (%)	76 (3)	53 (7)|	71 (3)	NA
Glucocorticoids	0	0	2	6
Analysis performed				
Northern	6	6	1	5
Immunostaining	6	6	3	7

* In 10 of these cases death was attributed to a fatal asthma attack.
† $p < 0.05$ *v* smokers, non-obstructed and COPD patients.
‡ All smokers were current smokers; all the rest were life time non-smokers except for three fatal asthma cases where ex-smoking status was uncertain.
§ $p < 0.01$ *v* the non-obstructed group.
¶ Mean of three patients. The fourth was a non-smoker.
\| $p < 0.001$ *v* the non-obstructed group.
** Data from three patients where lung functions were available.
FEV_1 = Forced expiratory volume in one second; FVC = forced vital capacity; NA = not available.

$TGF\text{-}\beta_1$ drives the abnormal synthesis of the extracellular matrix previously described in patients with asthma and COPD.

Methods

PATIENTS

The source of the tissue that formed the starting material for these studies is summarised in table 1. Twelve smokers undergoing lung resection for non-small cell carcinoma were part of an ongoing study of lung structure and function in this laboratory.[8] This group was subdivided into non-obstructed smokers (n = 6) and patients with COPD (n = 6) according to a preoperative FEV_1 above or below 80% of predicted, respectively. Lung tissue from these subjects was analysed by both Northern blotting and immunostaining. Four additional patients with COPD were selected for Northern analysis of peripheral lung *versus* segmental airways (not included in the table). For the asthmatic patients, because of the limited material available, simultaneous Northern analysis and immunohistochemical measurement was not feasible. Surgical specimens were obtained from four asthmatic patients with mild to moderate disease who required lung resection for non-small cell carcinoma (n = 3) or a vascular malformation. Twelve necropsy cases, including 10 fatal asthma attacks, were also studied by Northern blotting (n = 5) or by immunostaining (n = 7). The diagnosis of asthma was based on the criteria published by the American Thoracic Society[24] and confirmed by examination of the pathological specimens. The histological criteria used to establish the pathological changes include those in the lumen (inflammatory mucus plugs), the airway epithelium (sloughing and goblet cell metaplasia), submucosa (vascular dilatation and inflammatory infiltrate dominated by eosinophils), and a markedly thickened smooth muscle layer. These changes also include a generalised increase in airway wall thickness compared with lungs of smokers.[25]

TISSUE PROCESSING

Lung specimens resected for non-small cell carcinoma from the 12 smokers with or without airflow obstruction, as well as from the three asthmatic patients, were processed as previously described[8] by inflating them with a cryoembedding medium diluted 1:1 in saline (OCT, Miles, Elkhart, Indiana, USA), and freezing them over liquid nitrogen. Randomised peripheral samples 1·5 cm in diameter were cut out from an area remote from tumour and kept frozen at −70°C until they were analysed. The three specimens from the asthmatic patients were used only for immunohistochemical analysis. The additional surgical sample from an asthmatic subject was frozen without cryoprotective and used for Northern analysis. Lung tissue from the necropsy cases obtained within 24 hours after the time of death were either frozen without cryoprotective agent at −70°C (n = 5) to be used for Northern analysis, or fixed in formalin and embedded in paraffin blocks (n = 7) for immunohistochemical analysis.

cDNA PROBES

The probe for human $TGF\text{-}\beta_1$ consists of nucleotides 991 to 2041 of the cDNA.[26] This DNA contains 688 bp of the *N*-terminal region of the $TGF\text{-}\beta_1$ precursor followed by the entire 336 bp coding sequence of the mature peptide. The rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe, a 1·2 kb cDNA fragment which cross hybridises to the human GAPDH mRNA, was used as an internal control for RNA loading.[27] The cDNAs were labelled with α-[^{32}P]dCTP by the random priming method[28] to a specific activity of 1×10^9 cpm/μg.

NORTHERN BLOT ANALYSIS

Total RNA was extracted from 500 mg of lung parenchyma or lung tissue mainly comprised of central airways by the guanidinium thiocyanate phenol method[29] using a tissue homogeniser (Polytron, Kinematica, Luzern, Switzerland). The RNA (20 μg) was separated by 0·7 mol/l formaldehyde, 1% agarose gel electrophoresis, and blotted onto nylon membranes (Hybond-N, Amersham, UK). After the nucleic acids were cross linked to the membranes by ultraviolet transillumination at 312 nm for four minutes, the filters were prehybridised four hours at 42°C in 0·75 mol/l NaCl, 50 mmol/l NaH_2PO_4, 5 mmol/l Na_2EDTA, 5 × Denhardt's solution (100 × Denhardt's = 2% BSA, 2% Ficoll 400, 2% polyvinylpyrrolidone), 0·5% (w/v) SDS, 50% (v/v) deionised formamide, 100 μg/ml yeast tRNA, and 10% (w/v) dextran sulphate. Hybridisation was performed for 18 hours under the same conditions with 1×10^6 cpm/ml 32-phosphorus labelled cDNA probe. The maximal stringency of the washes after hybridisation reached 0·1 × SSC (20 × SSC = 3 mol/l NaCl, 0·3 mol/l sodium citrate, pH 7·0), 0·1% SDS at 65°C. The filters were used to expose to Agfa Curix Rp1 film (D) in the presence of an intensifying screen. The autoradiographic signal was quantified with the Ultroscan laser densitometer at 633 nm in the one dimensional mode and the GSXL

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analysis software v.2.1 (Pharmacia LKB, Uppsala, Sweden). Care was taken to obtain film exposures in the linear range of the emulsion – that is, below 1·4 absorbance units. The filters were successively probed with TGF-β_1 and GAPDH cDNAs. To correct for unequal loading, data were expressed as the density of the TGF-β_1 mRNA band divided by the density of the corresponding GAPDH mRNA band. GAPDH was chosen as an internal reference among other constitutive genes because its expression is not affected by TGF-β_1 activity.[30]

ANTIBODIES

The antibodies to TGF-β_1 used in our study, anti-LC (1–30) and anti-CC (1–30), were generous gifts from Drs K Flanders (National Institutes of Health, Bethesda, Maryland, USA) and L Ellingsworth (Celtrix Corporation, Palo Alto, California, USA), respectively. Both are polyclonal antibodies raised in rabbits against two different synthetic preparations of the same peptide sequence, corresponding to the first 30 amino acids of the *N*-terminal region of the mature TGF-β_1.[31–33] Anti-CC (1–30) principally stains the extracellular matrix-bound TGF-β_1 while anti-LC (1–30), according to previous reports, stains the intracellular sites of TGF-β_1 synthesis.[31 33 34] Anti-LC (1–30) was used on cryostat sections, while anti-CC (1–30) was applied to formalin fixed tissue.

IMMUNOHISTOCHEMISTRY

As the necropsy lung tissue used for Northern analysis was frozen without a cryoprotective agent, the morphology was not preserved well enough for immunohistochemical studies. Other asthma cases were therefore studied, as outlined above. For anti-LC (1–30) staining cryostat sections, 6 μm thick, were placed on poly-L-lysine coated slides and briefly fixed in cold acetone. In the case of anti-CC (1–30) staining formalin fixed tissue embedded in paraffin, cut as 4 μm thick sections, was dried at 37°C overnight, deparaffinised and hydrated in sequential gradients of ethanol. Both paraffin and cryosections were immersed in 0·6% hydrogen peroxide (Sigma Chemicals, St Louis, Missouri, USA) in methanol for one hour to block endogenous peroxidase activity. For staining with anti-LC (1–30) the sections were incubated with 1 mg/ml hyaluronidase (Sigma) in 0·1 mol/l sodium acetate buffer, pH 5·5, at 37°C for 30 minutes, washed in distilled water, and then incubated with 5% skim milk powder for 30 minutes. The milk was drained off and the sections were covered with anti-LC (1–30) at a final concentration of 8·35 μg/ml, then with Parafilm, and incubated at 20°C. After two hours the slides were gently rinsed in Tris buffer and incubated with biotinylated goat antirabbit secondary antibody (Vector Laboratories, Burlingame, California, USA) at 20°C for 30 minutes. After washing the sections were returned to the humid chamber for incubation with peroxidase labelled streptavidin (Biocan Scientific, Westgrove, Pennsylvania, USA) at 20°C for 80 minutes. The slides were then stained with freshly prepared 3-amino-9-ethyl carbazole (Sigma) at 0·263 mg/ml for 10 minutes, counterstained with Meyer's haematoxylin, mounted with CrystalMount (Biomeda Corp., Foster City, California, USA), and coverslipped. For staining with anti-CC (1–30) the same procedure was followed, except the hyaluronidase digestion was omitted and the primary antibody was used at a final concentration of 14 μg/ml. For negative controls the primary antibody was either replaced by non-immune serum or, in case of anti-LC (1–30), preincubated with TGF-β_1 (1–30) blocking peptide (generous gift from Dr I Clark-Lewis, Biomedical Research Centre, Vancouver) at 15 μg/ml. The pattern of staining was recorded and compared with that of a positive tissue control of known intensity of staining (a paraneoplastic stromal reaction) which was included with every run. The samples were graded on a scale of − to + + +, according to the relative intensity of staining of each tissue.

DATA ANALYSIS

Because of the small sample size and the non-normal distribution of the data, the non-parametric Mann-Whitney and Kruskal-Wallis tests were used when required. In order to compare the results from the two Northern blots (fig 1) the samples from the non-obstructed smokers were run on both gels. Individual ratios of the optical density of the TGF-β_1 band to that of the corresponding GAPDH band were then expressed as the percentage of the mean ratio measured for the non-obstructed control group on each respective blot. The same standardisation to the mean ratio of the control group was also used when comparing necropsy specimens with surgical specimens, and fatal with non-fatal asthma. In the Northern analysis of the central airways and the peripheral lung (fig 2) the mean ratio of the optical densities of the TGF-β_1 band to that of the corresponding GAPDH band lane were compared. Unless otherwise specified results are expressed as mean (SD). A p value < 0.05 was considered significant.

Results

Table 1 summarises the clinical data of the patients studied. The asthmatic lungs obtained at necropsy were, on average, from younger patients than the other groups. For the asthmatic patients the results of recent pulmonary function tests were available for only three of the cases where the material was obtained by surgical resection. In the necropsy group the cause of death was attributed to an asthma attack in 10 cases, the other two being a subarachnoid haemorrhage and coronary artery disease. Inhaled or oral glucocorticoids were known to be used by six of the 16 asthmatic patients and by none of the non-obstructed patients or those with COPD. Only three of the asthmatic patients were known to be active smokers.

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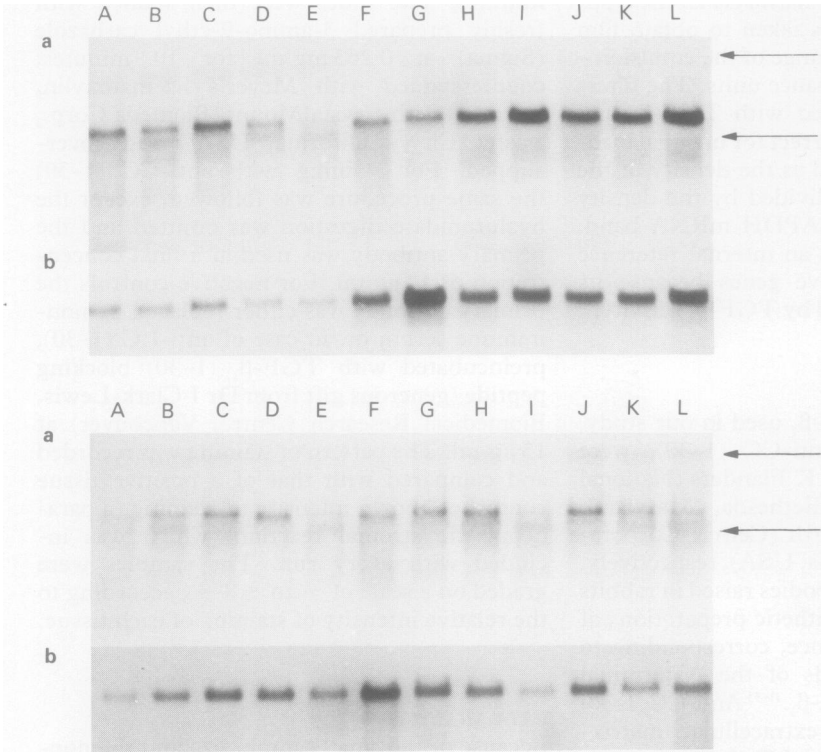

Figure 1 Top: Northern blot analysis from total RNA extracted from peripheral lung of non-obstructed controls (lanes A–F) and of asthmatics (lanes G–L). Lanes G, H, and K represent material from fatal asthma cases; lanes I and L represent necropsy specimens from non-fatal asthma cases, and lanes A–F and J represent material from surgical specimens. In panel (a) a single band of ~2·5 kb is detected with a TGF-β_1 cDNA probe. The arrows indicate the location of the 28S and the 18S ribosomal RNA. Exposure: 40 hours. In panel (b) the membrane was stripped and reprobed with a GAPDH cDNA probe as a marker of relative RNA loading. Exposure: 24 hours. Bottom: Northern blot analysis from total RNA extracted from peripheral lungs of non-obstructed controls (lanes A–F) and of patients with COPD (lanes G–L). In panel (a) the cDNA probe for TGF-β_1 detects the ~2·5 kb mRNA in all 12 samples. The arrows indicate the location of the 28S and the 18S ribosomal RNA. Some non-specific binding with the 18S ribosomal RNA is observed. Exposure: 48 hours. In panel (b) the membrane was reprobed with a GAPDH cDNA probe as in the top panel. Exposure: 18 hours.

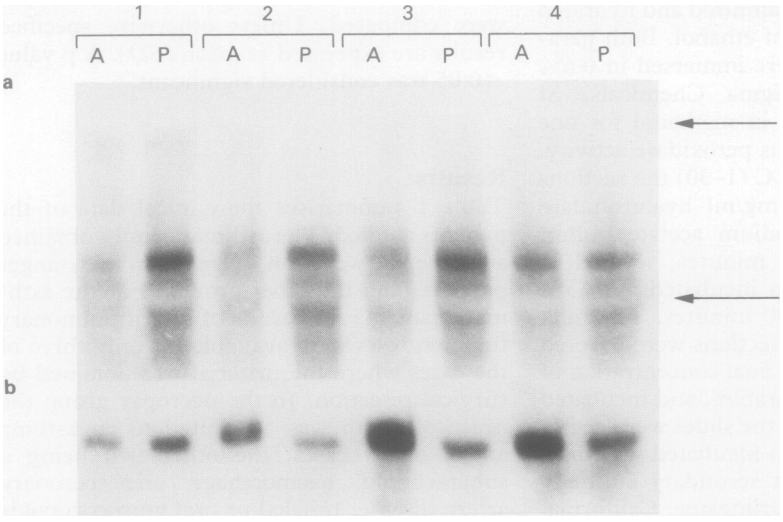

Figure 2 Total RNA from peripheral lung (P) and from segmental airway (A) was isolated from four additional patients with COPD. In panel (a) the TGF-β_1 mRNA signal is seen in all specimens, but with a stronger intensity in the peripheral lung than in large airways. The arrows indicate the location of the 28S and the 18S ribosomal RNA. Exposure: 72 hours. In panel (b) the same filter probed for GAPDH mRNA. Exposure: 24 hours.

NORTHERN ANALYSIS

The expected 2·5 kb band corresponding to TGF-β_1 mRNA[26] was detected in the 18 samples of peripheral lungs analysed (fig 1). Crossreactivity with either TGF-β_2 or TGF-β_3 subtypes was not observed, as the single signal detected did not correspond to the expected size of TGF-β_2 or TGF-β_3 mRNA. No major RNA degradation was seen as judged by ethidium bromide staining of the ribosomal RNA bands (data not shown) or on the autoradiographic signal from the TGF-β_1 and GAPDH mRNA bands (fig 1). The quality of the mRNAs from the necropsy cases, of which three were asthma deaths, appeared indistinguishable from those obtained from surgical specimens. This was confirmed by the densitometric analysis of the intensity of the autoradiographic signals (fig 1, top panel) resulting in a mean (SD) of 108% (48%) for the necropsy group ($n=5$) *versus* 101% (45%) for the surgical specimens ($n=7$, $p>0{\cdot}05$).

Evaluation of TGF-β_1 mRNA expression by densitometric analysis in the non-obstructed smokers (100% (49%)), patients with COPD (122% (33%)), and the asthmatic subjects (108% (43%)), after correction for unequal loading, showed no significant difference between these groups. To evaluate if the severity of the disease was correlated with the level of TGF-β_1 mRNA, samples from the three fatal asthma cases were selected and compared with the three specimens from patients with mild to moderate non-fatal asthma. RNA expression was the same in the fatal asthma and in the non-fatal cases (96% (57%) *versus* 121% (31%), $p>0{\cdot}05$). To analyse TGF-β_1 mRNA expression in different areas of the lung RNA from central airways (lobar, segmental, or subsegmental bronchi) and lung parenchyma was extracted from another four patients with mild COPD (fig 2). TGF-β_1 message was found in all eight samples and appeared to be more abundant in the peripheral lung in all four cases with a mean density of 1·107 (0·824) than in the airways with a mean of 0·418 (0·107) ($p<0{\cdot}01$).

IMMUNOHISTOCHEMISTRY

Immunostaining was analysed in airways with basement membrane lengths from 2·8 mm to 25 mm or shortest diameters of 0·2 to 5 mm. With the anti-TGF-β_1 LC (1–30), immunostaining on cryostat sections detected the presence of the TGF-β_1 precursor molecule in the bronchiolar epithelium, the submucosa, and in the airway smooth muscle (fig 3a and table 2). At a higher magnification the staining was observed to be more intense in the apical than the basolateral domain of the bronchial epithelial cell. In the peripheral lung the alveolar epithelium was not stained (fig 3b). Alveolar macrophages were usually positive but showed some heterogeneity in the intensity of staining (fig 3b). The blood vessels of the lung were also stained.

A second antibody, anti-TGF-β_1 CC (1–30), which detects the mature and extracellular form of TGF-β_1, was used on formalin fixed

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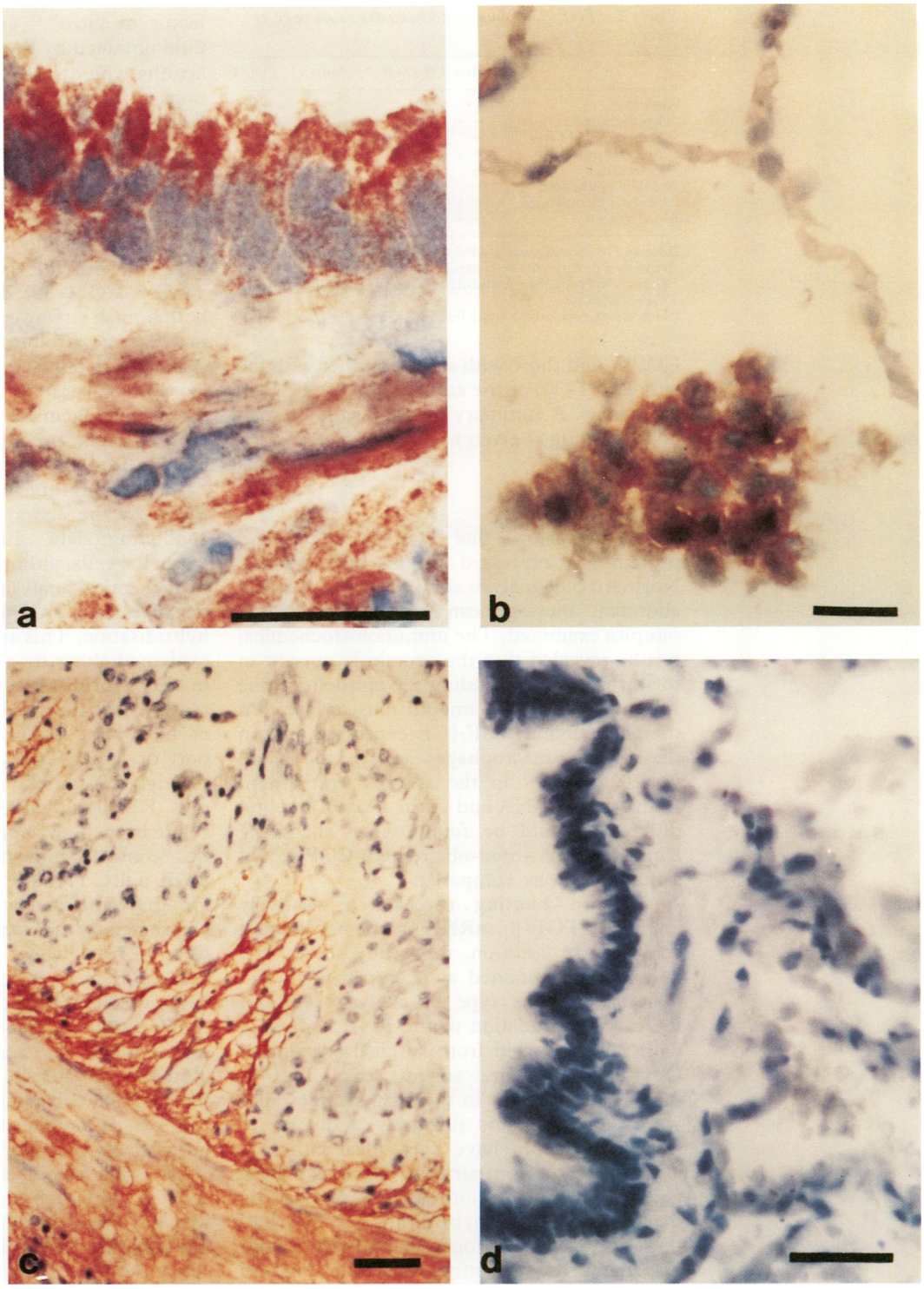

Figure 3 Immunohistochemical detection of TGF-β_1 (bar = 40 μm). (a) Bronchiole from an asthmatic patient stained with the anti-LC (1–30) antibody. Red positive staining is observed in the apex of the epithelial cells and in the smooth muscle. (b) Lung parenchyma from a patient with COPD incubated with anti-LC (1–30). Alveolar macrophages are strongly positive whereas the alveolar epithelium is not stained. (c) Medium sized bronchus from an asthmatic patient stained with the anti-CC (1–30). The bronchial epithelium is not stained. Strong reactivity is observed in the smooth muscle and in the connective tissue lying subjacent to the basement membrane. The basement membrane itself is not stained by the antibody. (d) Negative control of a small bronchiole where the primary antibody has been replaced by non-immune goat serum showing no reactivity. All sections are counterstained with haematoxylin.

tissue. Almost no reactivity was found in the airway epithelium (fig 3c). Staining in the airway wall was observed at the site of extracellular matrix deposition and was especially intense in the submucosa and the smooth muscle (fig 3c). Examination of lung parenchyma showed that, in contrast to the staining with anti-TGF-β_1 LC (1–30) antibody, the alveolar macrophages were not stained by anti-CC (1–30). The alveolar epithelium was also negative. Negative controls showed minimal non-specific staining of the epithelial basement membrane and the submucosa (fig 3d). There was no difference in the pattern of reactivity and in the overall intensity of staining between the three clinical groups (control, patients with

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Table 2 TGF-β_1 immunohistochemistry: summary of results

	Anti-LC (1–30)*	Anti-CC (1–30)†
Airway		
Epithelium	+ +	±
Subepithelial layer	+	+ + +
Smooth muscle	+ + +	+ +
Adventitia	±	+
Alveolar epithelium	−	−
Alveolar macrophages	+ +	−
Blood vessels	+ +	+ +

Intensity of staining was graded from − to + + + by comparison with a positive tissue control.
* Detects the precursor molecule and stains intracellular sites of TGF-β_1 synthesis.[31 33 34]
† Detects extracellular matrix bound TGF-β_1.[31 33 34]

COPD, and those with asthma) either with the anti-LC (1–30) or the anti-CC (1–30) TGF-β_1 antibodies. A summary of the results of antibody staining is given in table 2.

Discussion

This study shows that TGF-β_1 mRNA and protein are expressed in the human lung. Northern blot analysis showed that a relatively abundant message can be detected in all samples examined. The immunohistochemical results revealed that the protein is present in the airway wall, including the epithelium, the submucosa, and the smooth muscle layer. In the parenchyma TGF-β_1 is expressed only in the alveolar macrophages and in the blood vessels, but not in the alveolar epithelium. Both at the mRNA and at the protein level no difference could be found when the three clinical groups – non-obstructed, COPD, and asthmatic – were compared.

Northern blotting revealed a relatively abundant TGF-β_1 mRNA in the 18 samples analysed. In addition, this study shows that human lung obtained at necropsy is a valid material for this type of analysis, without increased degradation of the RNA compared with that extracted from surgical specimens. Our data therefore suggest that TGF-β_1 mRNA is abundant in the lung samples analysed. As asthma is primarily a disease of airways it would have been ideal either to isolate mRNA from airways tissue excluding parenchyma, or to localise the mRNA by quantitative in situ hybridisation. Neither of these approaches was open to us and we recognise that we are presenting data from a mixture of airways and parenchyma. As both bronchoalveolar lavage and biopsy studies have shown that the changes in asthma are present in most peripheral airways,[35] it may be quite difficult to separate airways from parenchyma in a satisfactory manner.

The two antibodies used in this study have been shown to recognise different epitopes of the TGF-β_1 molecule, the anti-TGF-β_1 LC (1–30) reacting with the peptide in the latent conformation, and the anti-TGF-β_1 CC (1–30) reacting only with the mature homodimer bound to the extracellular matrix.[33 34] It has been assumed, therefore, that the anti-TGF-β_1 LC (1–30) staining reflects the site where this growth factor is being synthesised.[31–33] Previous studies have shown that human alveolar macrophages are able to synthesise this growth factor in vitro[36 37] and that epithelial lining fluid obtained by bronchoalveolar lavage from healthy volunteers contains high concentrations of TGF-β_1.[38] The cell types involved in the synthesis of this growth factor in vivo were not identified in this last study. Our results extend these findings by showing that the airway epithelium and the alveolar macrophages stain positively with the anti-TGF-β_1 LC (1–30), while both are predominantly negative with the extracellular marker anti-TGF-β_1 CC (1–30). These results suggest that both human airway epithelial cells and alveolar macrophages produce the latent form of TGF-β_1 in vivo. Upon secretion into the extracellular space this latent form can be activated if the ad hoc chemical or enzymatic conditions are present to process the precursor into the mature form.

In the literature the study of TGF-β_1 synthesis by the airway epithelium has yielded conflicting data. In mouse airways[39] the epithelial cells, although positive by immunohistochemical analysis, have been found to be negative for TGF-β_1 mRNA by in situ hybridisation. This apparent paradox could be explained either by a lack of sensitivity of the in situ hybridisation technique or by a cross reactivity of the antibody with other subtypes of TGF-β or, less likely, by a selective transport of latent TGF-β_1 from the submucosa through the epithelium. Contrary to our findings, Khalil and colleagues found no staining of the bronchiolar epithelium from non-fibrotic "control" human lungs using the anti-TGF-β LC (1–30).[40] A possible explanation for this discrepancy is that the six non-obstructed control lungs analysed in our study were all from heavy smokers with non-small cell carcinomas. Although only non-tumour tissue was used in our study and no functional smoke induced abnormalities were found in these patients, one cannot exclude an abnormal induction of TGF-β_1 synthesis in the lungs of these individuals compared with true healthy controls. It should also be noted that in the initial characterisation of the anti-TGF-β_1 LC (1–30) some cross reactivity with TGF-β_2 or TGF-β_3, although absent in ELISA studies, was not ruled out in immunohistochemical applications.[31] Despite these conflicting results, one aspect of our present study is concordant with the two previous reports using the same antisera on human lung, namely, that the alveolar epithelium does not stain with anti-LC (1–30) antibody in control specimens.[34 40] Together with the absence of staining in our negative controls, we therefore feel confident that the positivity of the airway epithelium for the latent form of TGF-β_1 in the non-obstructed lungs is genuine.

Both at the mRNA and at the protein level we were unable to find any difference in TGF-β_1 expression in lungs from asthmatic and COPD patients compared with non-obstructed lungs. A clear limitation of this study is the small number of patients and the heterogeneity of the asthmatic group. Despite this difficulty, because of the rarity of asthmatic tissue available, surveys such as this one

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are important since animal models never fulfil all the pathological features of human asthma. If TGF-β_1 was involved in the pathogenesis of asthma, it would presumably be found in higher amounts in the more severe cases than in the mild ones. When we compared the mRNA levels from three fatal asthma cases with those of others with milder disease, however, no difference was observed. Drug treatment, especially with corticosteroids, could be a confounding factor in the analysis of these data. The reported effects of corticosteroids on TGF-β_1 gene expression in vitro differ according to the cell type studied, with either an induction,[41] a repression,[42] or no detectable change.[43] Limited evidence from studies in situ suggests, however, that in humans corticosteroid treatment has little effect on TGF-β_1 mRNA expression in the lung.[34 44] In our study the eight asthmatic patients known to be receiving either inhaled or oral corticosteroids did not differ from the other subjects in either their mRNA level or pattern of immunostaining for TGF-β_1.

Although increased expression of the TGF-β_1 gene was not shown in the cases of asthma and COPD reported here, the role of TGF-β_1 in the pathogenesis of these conditions cannot be definitively ruled out. Many regulatory steps at the post translational level are known to control TGF-β activity, notably the activation of the latent precursor,[22 23] the binding to extracellular matrix components or to soluble receptors,[45] as well as the inactivation through β_2-macroglobulin binding.[46] One can therefore speculate that, in the airway microenvironment, local conditions might increase TGF-β_1 bioactivity through one or several of these mechanisms. In addition, the potential involvement of other members of the TGF-β family, notably TGF-β_2 and TGF-β_3, remains to be ascertained. Finally, the recent cloning of the genes for type I and II receptors will facilitate studies to determine if the expression of these genes plays a regulatory role in controlling TGF-β_1 activity in vivo.[18 20]

In conclusion we have shown that TGF-β_1 is expressed in the human lung both at the mRNA and at the protein level. TGF-β_1 is localised mainly in the airway wall, including its epithelium, and in the alveolar macrophages. It does not appear to be overexpressed in the lungs of asthmatic and COPD patients. The subepithelial connective tissue deposition which is characteristic of asthmatic airways is therefore probably not the consequence of an abnormal synthesis of this growth factor.

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