Clin Exp Immunol 1996; **106**:114–119

Release of transforming growth factor-beta (TGF-β) and fibronectin by alveolar macrophages in airway diseases

A. M. VIGNOLA*, P. CHANEZ†, G. CHIAPPARA*, A. MERENDINO*‡, E. ZINNANTI*, J. BOUSQUET"", V. BELLIA & G. BONSIGNORE*‡ *Istituto di Fisiopatologia Respiratoria, C.N.R., Palermo, Italy, †Clinique des Maladies Respiratoires and CJF 92-10 INSERM, Hôpital Arnaud de Villeneuve, Montpellier, France, and ‡Clinica di Malatie Respiratorie, Universita di Palermo, Palermo, Italy*

(Accepted for publication 10 June 1996)

SUMMARY

Asthma and chronic bronchitis are associated with airway remodelling, and airway macrophages are present in bronchial inflammation. TGF-β and fibronectin released by alveolar macrophages possess a fibrogenic potency. The potential role of alveolar macrophages in airway remodelling was studied in asthma and chronic bronchitis by the release of TGF-β and fibronectin. Alveolar macrophages were isolated by bronchoalveolar lavage in 14 control subjects, 14 asthmatics and 14 chronic bronchitics. The spontaneous and lipopolysaccharide (LPS)- or concanavalin A (Con A)-induced release of TGF-β and fibronectin was measured by ELISA. Alveolar macrophages from chronic bronchitics spontaneously release greater amounts of TGF-β and fibronectin than those from asthmatic and control subjects. Alveolar macrophages from asthmatics release greater amounts of TGF-β and fibronectin than those from control subjects. The spontaneous release of TGF-β is significantly correlated with that of fibronectin. Fibronectin release was significantly reduced after LPS stimulation, and TGF-β release was significantly increased after LPS stimulation, except in chronic bronchitis patients. Con A increased the release of TGF-β in cells from normal subjects. This study suggests that activated macrophages play a role in airway remodelling in chronic bronchitis and to a lesser extent in asthma.

Keywords remodelling transforming growth factor-beta fibronectin asthma chronic bronchitis alveolar macrophages

INTRODUCTION

Chronic inflammation is a feature of many diseases of the airways. It is invariably associated with healing, which begins very early in the inflammation process. Healing results in repair and the replacement of dead or damaged cells by viable cells. Repair usually involves two distinct processes: regeneration which is the replacement of injured tissue by parenchymal cells of the same type, and replacement by connective tissue and its eventual maturation into scar tissue [1]. In many instances both processes contribute to the healing response. A chronic inflammatory disease of the airways is often associated with airway remodelling, including a wide variety of pathologic defects from partial restitution of organ structure and function to fibrosis leading to some component of airflow obstruction.

Asthma is a chronic inflammatory disease associated with remodelling of the airways [2], including subepithelial fibrosis [3]. Chronic bronchitis is another chronic obstructive disease of the airways in which inflammation is followed by fibrosis with extracellular matrix (ECM) deposition on the airways wall [4]. Growth factors such as TGF-β and glycoproteins of the ECM, such as fibronectin (FN), have been implicated in airway remodelling and pulmonary fibrosis [5].

The TGF-β family can be generated by a number of different cell types, including macrophages [6]. TGF-β is considered to be a major fibrogenic cytokine [6,7] and has been implicated in the generation of pulmonary fibrosis in humans [5]. In rats with pulmonary fibrosis induced by the administration of bleomicyn, total TGF-β1 content was several times higher than in normal rats, and increased production of TGF-β1 preceded the synthesis of ECM components [8].

FN, produced by a variety of cell types, is involved in cell attachment and chemotaxis [9] as well as in repair processes. The abnormal synthesis and accumulation of FN has already been demonstrated to be an important feature of fibrotic lesions in pulmonary fibrosis [9,10]. Deposits of FN beneath the basement membrane have been observed in asthma [11]. Moreover, it has been shown that the release of FN in the lung is modulated by TGF-β [9] and that the increased production of TGF-β precedes the synthesis of FN [8].

Correspondence: Antonio M. Vignola, Istituto di Fisiopatologia Respiratoria, C. N. R., Via Trabucco, Palermo, Italy.

Macrophages play a fundamental role in chronic inflammation, healing and repair processes. Airway macrophages have been implicated in the chronic inflammation both in asthma and chronic bronchitis [12,13]. In smokers, alveolar macrophages (AM) release high levels of mediators which are involved in lung injury such as elastase, ECM-degrading enzymes, plasminogen activator and oxygen free radicals [14]. Although the contribution of AM to healing and repair processes has been widely investigated in the development of fibrosing airways diseases [15,16], the level of macrophage involvement in the remodelling processes in asthma and chronic bronchitis remains unclear.

Since TGF-β and FN play a crucial role in tissue remodelling, and their increased release by AM has been considered as an important mechanism in the pathogenesis of lung fibrosis, a study was undertaken involving 14 normal subjects, 14 asthmatic patients and 14 chronic bronchitis patients to evaluate whether AM recovered from asthmatic and chronic bronchitis patients could contribute to airway remodelling by releasing enhanced levels of TGF-β1 and FN, either spontaneously or after stimulation with lipopolysaccharide (LPS) or concanavalin A (Con A).

SUBJECTS AND METHODS

Subjects

Fourteen asthmatics subjects (21–55 years, median 35 years) were studied. Asthma was defined as previously described [17] and all patients had a reversible airways disease. The severity of asthma, assessed using the clinical score of Aas, varied from mild to severe (1–4) and forced expiratory volume in 1 s (FEV_1) ranged from 50% to 90% of predicted values (median and 25–75% percentiles: 72, 62–80%). None of these subjects was a smoker. None had had any bronchial infection during the previous month and anti-inflammatory drugs had been stopped for an appropriate time [17]. In particular, none of the patients had been under corticosteroids of any form for at least 1 month.

Fourteen patients with chronic bronchitis (51–65 years, median 55 years) were studied. Chronic bronchitis was defined as previously described following the criteria of the American Thoracic Society [18]. Patients were excluded if they had a history of allergic diseases, wheezing, an improvement in the FEV_1 of more than 12% after inhalation of 200 μg of salbutamol**,** or if they had had a bronchial infection during the month preceding the study. They were all smokers, and none of the patients had been under corticosteroids of any form for at least 1 month.

Fourteen healthy subjects (20–52 years, median 34 years) were used as a control group. None of these subjects had ever smoked. None had had any bronchial infection during the previous month.

Informed consent was obtained from all subjects prior to the study, which was approved by the Ethics Committee of our hospital.

Recovery of AM

Pulmonary cells were obtained by bronchoalveolar lavage (BAL) as previously described [19]. Five to seven aliquots of 50 ml of sterile and isotonic saline were injected and gently aspirated with a syringe. The BAL fluid was immediately centrifuged (400 ***g***, 4°C, 20 min) and the cell pellet used for subsequent study. Cells were counted using a haemocytometer and differential cell counts were performed with the use of Kimura's stain. Viability was assessed by exclusion of trypan blue stain. After cytocentrifugation using a cytocentrifuge, fixed cell preparations were stained with May–Grünwald–Giemsa (MGG) to confirm differential cell counts. BAL cells were resuspended at a concentration of 5×10^5/ml in RPMI with 10% fetal calf serum (FCS) and were allowed to adhere onto plastic dishes for 2 h in an incubator with a 5% CO_2 moist atmosphere. After adherence, purity of the cells was assessed by immunohistochemistry using the pan-macrophage MoAb anti-CD-68 (Dako,Versailles, France).

Activation of cells

Non-adherent cells from BAL were removed by three gentle washings, and remaining cells were maintained under the same environmental conditions overnight. Cells were incubated for 24 h in the presence or absence of LPS or Con A. These two stimulants were chosen since they had previously been shown to induce the release of both TGF-β and FN [20]. The supernatants were then recovered and stored at −20°C until measurements of TGF-β1 and FN were performed. Viability of the cells was assessed by exclusion of trypan blue stain.

Dose– and time–response relationship

The optimal dose of LPS and Con A was studied using dose–response curves in four subjects in each group (1–10 μM LPS or Con A for 24 h at 37°C). Time–response curves were performed over a period of 48 h in two experiments using a LPS or Con A concentration of 10 μM.

Measurement of active TGF-β1 in supernatants

TGF-β1 was measured in the supernatant fluids by ELISA (Predicta TGF-β1 Kit; Genzyme, Cambridge, MA). In order to measure biologically active TGF-β1, samples were acidified using HCl for 1 h [21]. After acidification the samples were neutralized to pH 7·0–7·4 with NaOH and measurements of TGF-β1 were performed immediately. The detection limit of the assay was 0·05 ng/ml.

Measurement of FN in supernatants

FN was measured in the supernatant according to the technique of Campbell *et al.* [22], using a highly specific indirect ELISA assay utilizing a sandwich method, on dishes coated with anti-FN antibodies. Antibodies and standards were obtained from Calbiochem (La Jolla, CA). The limit of detection was 50 pg/ml.

Statistical analysis

Non-parametric tests were used. The Kruskall–Wallis test was used to compare the three groups. The Mann–Whitney *U*-test was used for unpaired comparisons and the Spearman rank test was used for correlations. For multiple correlations, the Bonferroni's correction was used. Results are given as medians and 25–75% percentiles.

RESULTS

Patients' characteristics

Four patients had mild asthma (Aas score 1–2), six moderate asthma (Aas score 3) and four severe asthma (Aas score 4). There was no significant difference between asthmatics and normal subjects in terms of age and sex. Seven patients with chronic bronchitis were considered as having chronic obstructive pulmonary diseases (COPD) ($FEV_1 < 70\%$ of predicted). Patients with chronic bronchitis were significantly older than

Table 1. Bronchoalveolar lavage cell content

	Controls	Asthma	Bronchitis
Macrophages (%)	86 ± 8	82 ± 11	79 ± 10
Lymphocytes (%)	12 ± 11	15 ± 13	4 ± 3
Eosinophils (%)	0	$2{\cdot}5 \pm 4$	$1{\cdot}5 \pm 1{\cdot}0$
Neutrophils (%)	1 ± 1	1 ± 2	14 ± 18

those with asthma ($P < 0{\cdot}03$, Mann–Whitney *U*-test) and control subjects ($P < 0{\cdot}03$, Mann–Whitney *U*-test).

The characteristics of the cellular content of the BAL fluid are presented in Table 1.

Spontaneous release (Figs 1, 2 and 3 and Table 2)

The purity of the cells, assessed after the adherence step, was very high, since $99 \pm 1\%$ (mean $\pm$ s.d.) were $CD68^+$.

In chronic bronchitis patients, the release of TGF-β1 and FN was significantly greater than the levels of release in asthmatic or control subjects. In asthmatic subjects, the release of TGF-β1 and FN was significantly greater than in control subjects. Finally, the spontaneous release of TGF-β1 by AM was significantly correlated with that of FN (Fig. 3).

Modulation of TGF-β and FN release by LPS and Con A

Dose–response relationship showed that for all three groups of patients the concentration inducing maximum release of both TGF-β and FN was 10 μM for both LPS and Con A (Fig. 4). The time–response relationship showed that the effects of LPS or Con A increased up to 24 h and then reached a plateau (data not shown).

LPS (10 μM) significantly increased the release of TGF-β1 by AM isolated from normal subjects and asthmatics after a 24-h period of incubation (Fig. 1, Table 1). Conversely, LPS did not significantly modulate the release of TGF-β by AM from chronic bronchitis patients. Con A (10 μM) was able to increase the release of TGF-β only by AM isolated from normal subjects, and had no effect on AM from asthmatic or chronic bronchitis patients.

The release of FN by AM isolated from normal, asthmatic and chronic bronchitis subjects was significantly decreased by LPS (10 μM) following incubation for 24 h, whilst addition of Con A had no significant effect on the release of this mediator (Fig. 2, Table 1).

DISCUSSION

This study shows that AM isolated by BAL from chronic bronchitis patients spontaneously release greater amounts of TGF-β1 and FN than those from asthmatic and control subjects. Of the two latter groups, AM from patients with asthma release greater amounts of TGF-β1 and FN than those from control subjects. The spontaneous release of TGF-β1 is significantly correlated with that of FN in all groups.

Human airway macrophages play a crucial role in the pathogenesis of fibrotic lung processes [15,20]. AM regulate healing and repair processes by releasing several growth factors and/or ECM components, amongst which TGF-β and FN have been deemed to be of importance [10]. Moreover, TGF-β and FN play a central role in modulating the damage and repair of lung tissues [10,23].

We have shown that AM from control subjects spontaneously release relatively low levels of TGF-β, while higher levels of this cytokine are released by AM from asthmatic subjects, and AM from patients with chronic bronchitis release the greatest levels of this cytokine. The antibodies used for measurement of TGF-β were selected to detect the biologically active form of this growth factor. Although the isolation of mononuclear cells by adherence is a routine procedure, the culture of cells overnight and adherence on plastic can activate them to release mediators spontaneously and to express mRNA for cytokines [24,25]. However, since the procedure was identical in asthmatics and normal subjects, it should affect all cells similarly. TGF-β is a growth factor involved in fibrosis, since it induces the deposition of ECM by simultaneously

Table 2. Release of TGF-β and fibronectin (FN) by alveolar macrophages

				P †		
	Controls	Asthma	Chronic bronchitis	C/A	C/BC	A/BC
TFG-β (ng/million cells)						
Baseline	210 (189–250)	435 (359–576)	540 (437–641)	0·005	0·002	NS
LPS	347 (389–370)	596 (493–987)	621 (513–742)	NS	0·01	NS
P LPS/baseline*	0·01	0·004	NS			
Con A	402 (389–476)	523 (383–723)	827 (646–964)	NS	0·0003	0·01
P Con A/baseline*	0·01	NS	NS			
FN (ng/million cells)						
Baseline	70 (50–100)	148 (120–173)	239 (181–350)	0·0007	0·0002	0·004
LPS	40 (38–52)	114 (109–155)	204 (168–300)	0·007	0·0002	0·001
P LPS/baseline*	0·02	0·03	0·03			
Con A	87 (84–100)	164 (132–177)	267 (201–344)	0·004	0·003	0·01
P Con A/baseline*	NS	NS	NS			

* Within group analysis: Wilcoxon *W*-test.

† Between group analysis: Mann–Whitney *U*-test with Bonferroni's correction.

Results expressed in medians and 25–75% percentiles.

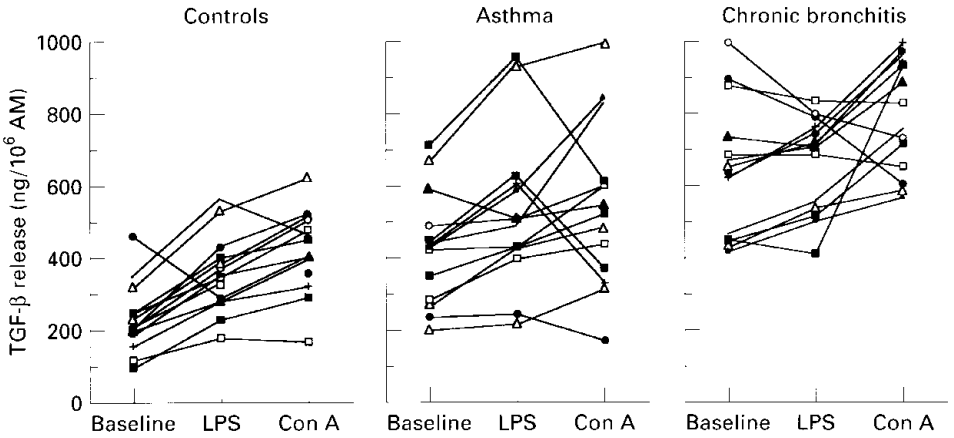

Fig. 1. Spontaneous and induced release of TGF-β by alveolar macrophages. LPS, Lipopolysaccharide; Con A, concanavalin A.

stimulating cells to increase the synthesis of most ECM proteins, decreasing the production of ECM-degrading proteases and increasing the production of protease inhibitors (TIMPs) [26]. In patients with fibrotic lung diseases, increased TGF-β production co-localized with areas of increased fibrotic ECM protein deposition has been demonstrated in biopsies [27] as well as in BAL cells. These findings suggest that expression of this growth factor results in an enhanced ECM synthesis and deposition in the disease process.

In this study, FN was found to be released in AM from control subjects, and to a greater extent in AM from patients with asthma and chronic bronchitis. FN is involved in cell attachment and migration and tissue repair [28], and its increased synthesis and accumulation within tissues was found to cause remodelling of the airway wall as shown in fibrotic diseases [27,29]. Moreover, in asthma the increased accumulation of FN within the bronchial mucosa has been identified as an important causative factor of the thickening of the basal membrane [3]. The release and expression of FN are directly modulated by TGF-β. Moreover, it has been found that increased TGF-β production precedes the synthesis of FN, suggesting a role for TGF-β in airway repair through modulation of FN production.

The results reported in this study show that both in asthma and chronic bronchitis, AM are an important source of biologically active TGF-β and FN. Thus, airways macrophages may play an active role in the regulation of mesenchymal cell proliferation and release of ECM components, leading to their deposition beneath the basal membrane, which represents a characteristic histopathological feature in asthma [3,30]. In addition, the putative role played by TGF-β1 in airway wall thickening in asthma has been recently proposed in a study showing that levels of TGF-β1 are increased in BAL fluid of asthmatic patients at baseline and after local allergen challenge [31]. In chronic bronchitis, airway wall fibrosis has also been demonstrated [32,33]. The elevated levels of TGF-β and FN released by AM from patients with chronic bronchitis may be of importance in the regulation of fibrosis, since airways macrophages are increased in numbers in the bronchial mucosa [13].

The modulation of the release of TGF-β and FN varies depending on the stimulus and the disease state. The results reported in this study show that LPS and Con A can modulate TGF-β release. It seems that cells that are up-regulated, as in the case of chronic bronchitis, cannot be further activated, and such findings have already been reported in AM of asthmatic patients [34]. In asthma, AM are in an intermediate state between cells of normal subjects and those of patients with chronic bronchitis, the latter being fully activated. The difference in activation patterns between LPS and Con A are not fully understood. The effects of LPS and Con A on FN release may be surprising, but the down-regulating effect of LPS on the release of FN has already been

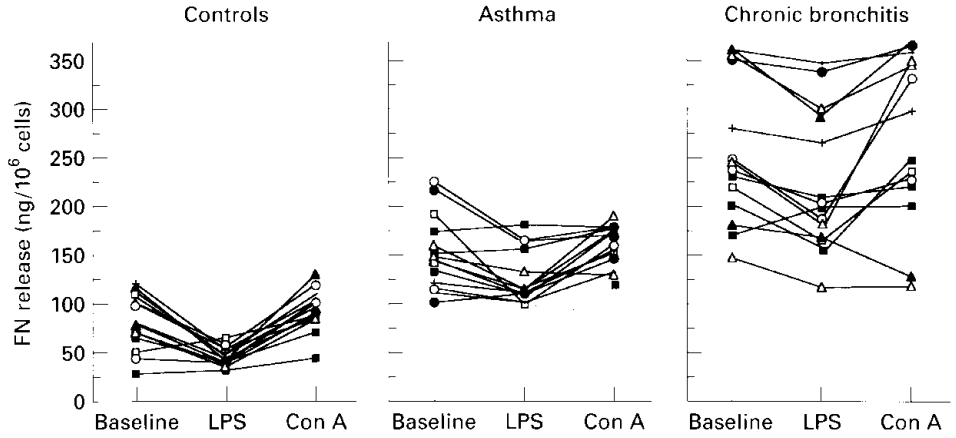

Fig. 2. Spontaneous and induced release of fibronectin (FN) by alveolar macrophages. LPS, Lipopolysaccharide; Con A, concanavalin A.

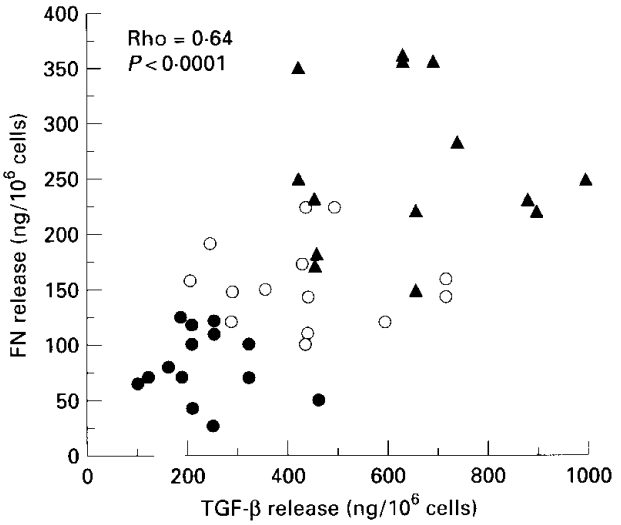

Fig. 3. Correlation between the spontaneous release of TGF-β and fibronectin (FN) by alveolar macrophages. Correlation by Spearman rank test. ●, Controls; ○, asthma; ▲, chronic bronchitis.

demonstrated using AM isolated from both normal subjects and patients with pulmonary fibrosis [35]. These data show that, although LPS is considered a potent monocyte-macrophage activator, it can also exert down-regulating effects according to the mediator studied as well as the activation state of the cells.

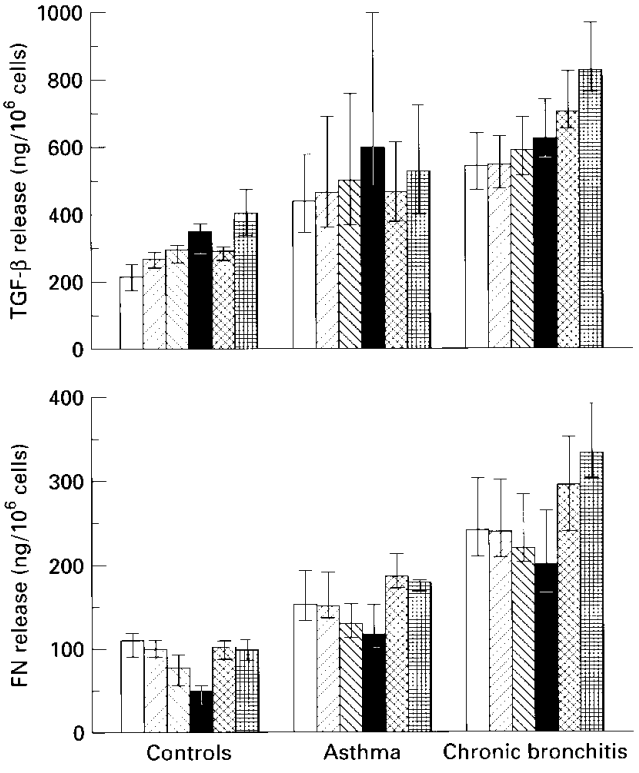

Fig. 4. Dose–response relationship of TGF-β and fibronectin (FN) release by alveolar macrophages. Results expressed in medians and 25–75 percentiles of four experiments done for each patient's group. □, Baseline; ▨, 1, lipopolysaccharide (LPS); ▧, 5, LPS; ■, 10, LPS; ▩, 5, concanavalin A (Con A); ▦, 10, Con A.

The increased ability of AM isolated from asthmatic and chronic bronchitis patients to release FN and TGF-β may be of importance in understanding the pathogenesis of airway remodelling associated with both diseases. AM have often been identified as a major source of mediators contributing to fibrotic alterations within the lung, and their role has been greatly emphasized in the pathogenesis of fibrotic lung diseases. The present study suggests a role for these cells in airway wall remodelling in chronic bronchitis, and to a lesser extent in asthma.

REFERENCES

1 Cotran R, Kumar V, Robin S. Inflammation and repair. In: Cotran R, Kumar V, Robi S, eds. Robbins pathologic basis of disease. Philadephia: WB Saunders, 1989:39–87.

2 Bousquet J, Chanez P, Lacoste JY *et al.* Asthma: a disease remodeling the airways. Allergy 1992; **47**:3–11.

3 Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. Lancet 1989; **1**:520–4.

4 Thurlbeck WM. Pathology of chronic airflow obstruction. Chest 1990; **97**:6S–10S.

5 Khalil N, O'Connor RN, Unruh HW *et al.* Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol 1991; **5**:155–62.

6 Border W, Noble N. Transforming growth factor β in tissue fibrosis. N Engl J Med 1994; **331**:1286–92.

7 Kovacs E, DiPietro L. Fibrogenic cytokines and connective tissue production. FASEB J 1994; **8**:854–61.

8 Westergren-Thorsson G, Hernnas J, Sarnstrand B, Oldberg A, Heinegard D, Malmstrom A. Altered expression of small proteoglycans, collagen, and transforming growth factor-beta 1 in developing bleomycin-induced pulmonary fibrosis in rats. J Clin Invest 1993; **92**:632–7.

9 Romberger DJ, Beckmann JD, Claassen L, Ertl RF, Rennard SI. Modulation of fibronectin production of bovine bronchial epithelial cells by transforming growth factor-beta. Am J Respir Cell Mol Biol 1992; **7**:149–55.

10 Rennard SI, Hunninghake GW, Bitterman PB, Crystal RG. Production of fibronectin by the human alveolar macrophage: mechanism for the recruitment of fibroblasts to sites of tissue injury in interstitial lung diseases. Proc Natl Acad Sci USA 1981; **78**:7147–51.

11 Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 1990; **3**:507–11.

12 Poston RN, Chanez P, Lacoste JY, Litchfield T, Lee TH, Bousquet J. Immunohistochemical characterization of the cellular infiltration in asthmatic bronchi. Am Rev Respir Dis 1992; **145**:918–21.

13 Saetta M, Di-Stefano A, Maestrelli P *et al.* Activated T-lymphocytes and macrophages in bronchial mucosa of subjects with chronic bronchitis. Am Rev Respir Dis 1993; **147**:301–6.

14 Sibille Y, Reynolds HY. Macrophages and polymorphonuclear neutrophils in lung defense and injury. Am Rev Respir Dis 1990; **141**:471–501.

15 Shaw R, Kelly J. Macrophages/monocytes. In: Phan S, Thrall R, eds. Pulmonary fibrosis. Vol. 80. New York: Marcel Dekker, 1995:405–44.

16 Brody AR. Control of lung fibroblast proliferation by macrophage-derived platelet-derived growth factor. Ann NY Acad Sci 1994; **725**:193–9.

17 Bousquet J, Chanez P, Lacoste JY *et al.* Eosinophilic inflammation in asthma. N Engl J Med 1990; **323**:1033–9.

18 Lacoste JY, Bousquet J, Chanez P *et al.* Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis, and chronic obstructive pulmonary disease. J Allergy Clin Immunol 1993; **92**:537–48.

19 Chanez P, Vago P, Demoly P *et al.* Airway macrophages from patients

with asthma do not proliferate. J Allergy Clin Immunol 1993; **92**:869–77.

20 Assoian RK, Fleurdelys BE, Stevenson HC *et al.* Expression and secretion of type beta transforming growth factor by activated human macrophages. Proc Natl Acad Sci USA 1987; **84**:6020–4.

21 Danielpour D. Improved sandwich enzyme-linked immunosorbent assays for transforming growth factor beta 1. J Immunol Methods 1993; **158**:17–25.

22 Campbell A, Vignola A, Chanez P *et al.* Functional assessment of viability of epithelial cells. Comparison of viability and mediator release in healthy subjects and asthmatics. Chest 1992; **101**:25S–27S.

23 Khalil N, Whitman C, Zuo L, Danielpour D, Greenberg A. Regulation of alveolar macrophage transforming growth factor-beta secretion by corticosteroids in bleomycin-induced pulmonary inflammation in the rat. J Clin Invest 1993; **92**:1812–8.

24 Kouzan S, Nolan RD, Fournier T, Bignon J, Eling TE, Brody AR. Stimulation of arachidonic acid metabolism by adherence of alveolar macrophages to a plastic substrate. Modulation by fetal bovine serum. Am Rev Respir Dis 1988; **137**:38–43.

25 Haskill S, Johnson C, Eierman D, Becker S, Warren K. Adherence induces selective mRNA expression of monocyte mediators and proto-oncogenes. J Immunol 1988; **140**:1690–4.

26 Eptein F. Transforming growth factor-β in pulmonary fibrosis. N Engl J Med 1984; **10**:1286–90.

27 Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci USA 1991; **88**:6642–6.

28 Shoji S, Ertl RF, Linder J, Romberger DJ, Rennard SI. Bronchial epithelial cells produce chemotactic activity for bronchial epithelial cells. Possible role for fibronectin in airway repair. Am Rev Respir Dis 1990; **141**:218–25.

29 Lazenby AJ, Crouch EC, McDonald JA, Kuhn CD. Remodeling of the lung in bleomycin-induced pulmonary fibrosis in the rat. An immuno-histochemical study of laminin, type IV collagen, and fibronectin. Am Rev Respir Dis 1990; **142**:206–14.

30 Saetta M, Maestrelli P, Di-Stefano A *et al.* Effect of cessation of exposure to toluene diisocyanate (TDI) on bronchial mucosa of subjects with TDI-induced asthma. Am Rev Respir Dis 1992; **145**:169–74.

31 Madden J, Redington A, Frew A, Djukanovic R, Holgate S, Howarth P. TGFβ in asthma: measurement in bronchoalveolar lavage: baseline and after local allergen challenge. Am J Respir Crit Care Med 1995; **151**:A703.

32 Cosio M, Ghezzo H, Hogg J *et al.* The relations between structural changes in small airways and pulmonary function tests. N Engl J Med 1977; **298**:1277–81.

33 Adesina AM, Vallyathan V, McQuillen EN, Weaver SO, Craighead JE. Bronchiolar inflammation and fibrosis associated with smoking. A morphologic cross-sectional population analysis. Am Rev Respir Dis 1991; **143**:144–9.

34 Chanez P, Vignola AM, Paul-Eugene N *et al.* Modulation by interleukin-4 of cytokine release from mononuclear phagocytes in asthma. J Allergy Clin Immunol 1994; **94**:997–1005.

35 Yamauchi K, Martinet Y, Crystal RG. Modulation of fibronectin gene expression in human mononuclear phagocytes. J Clin Invest 1987; **80**:1720–7.