Transforming Growth Factor β_1 and Recruitment of Macrophages and Mast Cells in Airways in Chronic Obstructive Pulmonary Disease

WILLEM I. de BOER, ANNEMARIE van SCHADEWIJK, JACOB K. SONT, HARI S. SHARMA, JAN STOLK, PIETER S. HIEMSTRA, and J. HAN J. M. van KRIEKEN

Departments of Pulmonology and Pathology, Leiden University Medical Center, Leiden, and Department of Pharmacology, Erasmus University, Rotterdam, The Netherlands

Chronic airways inflammation is one of the features of chronic obstructive pulmonary disease (COPD). We demonstrated previously that bronchiolar epithelium in COPD contains increased numbers of macrophages and mast cells. Transforming growth factor β_1 (TGF-β_1) may be involved in this influx because it has chemotactic activity for macrophages and mast cells. In this study, we examined expression patterns of TGF-β_1, TGF-β receptors type I and II (TGF-βRI and TGF-βRII) by immunohistochemistry and mRNA *in situ* hybridization in peripheral lung tissue of 14 current or ex-smokers with COPD ($FEV_1 < 75\%$) and 14 without COPD ($FEV_1 > 84\%$). In both groups, TGF-β_1 and its receptors are present in airway and alveolar epithelial cells, airway and vascular smooth muscle cells, and tissue and alveolar $CD68^+$ cells (considered herein to be macrophages). In subjects with COPD, a semiquantitative analysis revealed approximately twofold higher levels of TGF-β_1 mRNA and protein in bronchiolar and alveolar epithelium ($p < 0.02$) as compared with subjects without COPD. With regard to bronchiolar epithelial cells, we found a significant correlation between TGF-β_1 mRNA and protein expression ($r = 0.62$; $p < 0.002$), and between the FEV_1 of all subjects together and TGF-β_1 protein ($r = -0.60$; $p < 0.0002$) and mRNA ($r = -0.67$; $p < 0.002$) levels. The epithelial expression of TGF-β_1 mRNA and TGF-β_1 protein correlates with the number of intraepithelial macrophages (both: $r = 0.44$; $p < 0.03$) whereas intraepithelial mast cell numbers correlate with epithelial TGF-β_1 mRNA expression. These data suggest a role for TGF-β_1 in recruiting macrophages into the airway epithelium in COPD. de Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, van Krieken JHJM. Transforming growth factor β_1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. AM J RESPIR CRIT CARE MED 1998;158:1951–1957.

Chronic obstructive pulmonary disease (COPD) comprises emphysema and chronic bronchitis/bronchiolitis with chronic airflow obstruction. One of the characteristics of COPD is a thickened bronchiolar airway wall accompanied by an influx of inflammatory cells, an increase in smooth muscle mass, and the deposition of extracellular matrix (1). Several studies demonstrated a higher number of macrophages, mast cells, or T lymphocytes, especially in the airway epithelium of patients with COPD (2–6). Other studies showed a modified deposition of extracellular matrix components including collagens, especially in the peripheral airways of patients with emphysema (7). This may include an increased collagen deposition and an altered structure of collagen fibers (7, 8). Yet, little is known about the molecular mechanisms underlying the pathogenesis of COPD.

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Correspondence and requests for reprints should be addressed to W. I. de Boer, Ph.D., Dept. of Pathology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

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Transforming growth factor beta 1 (TGF-β_1) is a multifunctional growth factor that modulates cellular proliferation and induces differentiation and synthesis of extracellular matrix proteins including collagens and fibronectin in many types of cells. TGF-β_1 also induces chemotaxis of inflammatory cells such as mononuclear phagocytes, mast cells, and T lymphocytes (9–12). Moreover, TGF-β_1 is reported to be the most potent mast cell chemoattractant among three other mast cell chemotactic proteins: interleukin-3 (IL-3), c-*kit* ligand, and laminin (12). Previous studies demonstrated that TGF-β_1 is expressed in various tissues including human lungs (13, 14). In subjects without COPD, TGF-β_1 protein and messenger RNA (mRNA) expression are reported within bronchial epithelial cells, alveolar macrophages, and smooth muscle cells (13–16). The TGF-β_1 protein localization in subepithelial cells and endothelial cells is not yet clear. Little is known about the pulmonary expression pattern of TGF-β or its receptors in subjects with COPD. Moreover, the expression data from three studies on COPD patients were conflicting, which may in part be due to a difference in the selection of patients (17–19).

In this study, we investigated the localization patterns of TGF-β_1 mRNA, and protein, as well as TGF-β receptors type I and II in pulmonary tissue from subjects with and without COPD. Previously, we demonstrated an increase in macro-

phages and mast cells in bronchiolar epithelium of current or ex-smokers with COPD compared with smokers or ex-smokers without COPD (6). Using sequential sections of the same tissue specimens, we found a significant increase in TGF-β_1 mRNA and protein levels in airway epithelial cells in subjects with COPD as compared with those without COPD. These expression levels correlated with the increased numbers of intraepithelial macrophages (6).

METHODS

Antibodies

Polyclonal rabbit anti-human antibodies raised against synthetic peptides corresponding to either amino acids 158–179 of transforming growth factor β receptor type I (TGF-βRI) or amino acids 550–565 of TGF-β receptor type II (TGF-βRII) (both within the carboxyl terminal domain), and their neutralizing control peptides were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Cell type specific monoclonal antibodies against CD3, CD68, and tryptase (AA1) and secondary antibodies were purchased from Dako (Glostrup, Denmark). The rabbit polyclonal anti-human TGF-β_1 antibody raised against a synthetic peptide corresponding to the C-terminal amino acids 371–390 of human TGF-β_1 was kindly donated by Dr. E. de Heer (Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands) and its use has previously been described (20, 21).

Subjects

For this study, we used lung tissue specimens of subjects with or without COPD (6). Briefly, we selected tissue specimens from peripheral airways (airway diameter ranging from 1 to 3 mm) from current or ex-smokers who underwent lobectomy or pneumectomy for lung cancer. Fourteen subjects with COPD ($FEV_1 < 75\%$ of predicted value before bronchodilatation; reversibility in the FEV_1 of $\leq 13\%$ of the predicted value after 400 μg inhaled salbutamol) were included, as well as 14 subjects without COPD (FEV_1 before bronchodilatation $> 84\%$ predicted). The total lung capacities (TLCs) were not below normal levels (TLC $\geq 80\%$ predicted). Exclusion criteria included: (*1*) diffuse pulmonary inflammation of fibrotic disorders; (*2*) absence of tumor-free or poststenotic pneumonia-free lung tissue specimens; and (*3*) obstruction of central bronchi due to the tumor. All patients lack upper respiratory tract infection and did not receive antibiotics perioperatively. All patients had not received glucocorticosteroids during 3 mo before resection, four patients received glucocorticosteroids only perioperatively (Table 1). Data on lung function tests of these patients are presented in Table 1 and are described previously (6). Subjects with COPD could not be subdivided into patients with either chronic bronchitis or emphysema alone.

TABLE 1

SUBJECT CHARACTERISTICS*

Case	Sex	Age	Pack-Years	FEV_1	FEV_1/FVC	Steroid
Non-COPD						
1	M	79	47	86	0.73	No
2	M	77	50	87	0.62	No
3	M	75	26	89	0.74	No
4	M	65	76	90	0.71	No
5	M	71	60	92	0.70	No
6	F	41	1	97	0.83	No
7	F	56	45	99	0.68	No
8	M	67	110	100	0.69	No
9	M	60	33	101	0.67	No
10	M	79		102	0.73	No
11	F	28	3	106	0.71	No
12	M	67	27	108	0.66	No
13	M	69	38	118	0.76	No
14	M	68	35	133	0.86	No
Mean ± SEM		64 ± 3.7	42 ± 7.7	101 ± 3.3	0.72 ± 0.02	
COPD						
15	M	58	55	48	0.38	Yes
16	M	65	68	50	0.38	No
17	M	76	43	55	0.51	Yes
18	M	52	21	57	0.50	Yes
19	M	57	38	61	0.58	No
20	M	75	34	63	0.65	No
21	M	71	38	63	0.63	No
22	M	66	45	66	0.43	Yes
23	M	48	68	66	0.51	No
24	M	72	58	69	0.61	No
25	M	65	19	69	0.52	No
26	M	60	43	70	0.55	No
27	M	71	44	70	0.66	No
28	M	53	47	72	0.63	No
Mean ± SEM		64 ± 2.3	44 ± 0.82	63 ± 2	0.54 ± 0.02	
p Value		0.84	0.82	5E-9	5E-6	

* FEV_1 and FVC are given as percentage of predicted values.

Immunohistochemistry

Serial paraffin-embedded tissue sections (3 μm thick) were alternately used for TGF-β_1 *in situ* hybridization and immunohistochemistry on TGF-β_1, TGF-βRI, and TGF-βRII. Detection of cell-specific markers on adjacent sections was performed in order to confirm the type of cell. Immunocytochemistry was performed on serial sections essentially as described earlier (6, 21). After deparaffination, endogenous peroxidase was inactivated with 0.3% hydrogen peroxide. Sections to be stained with anti-CD68 were treated with proteinase K. Subsequently, sections were preincubated with 1% (wt/vol) bovine serum albumin (BSA). Antigen expression was demonstrated with appropriate dilutions of the primary antibodies in conjugated immunoenzyme assays using a secondary biotin-conjugated antibody and a tertiary complex of streptavidin–avidin–biotin conjugated to horseradish peroxidase, and 3-amino-9-ethyl-carbazole (AEC) as chromogen. Finally, the sections were counterstained with Mayer's hematoxylin. Incubation with phosphate-buffered saline (PBS) supplemented with 1% BSA instead of the primary antibody served as a negative control.

The possibility of false-positivity with anti-TGF-βRI and anti-TGF-βRII antibodies was verified by preabsorption of the first antibodies with their specific neutralizing control peptides following the manufacturer's instructions, and found to be negative.

The TGF-β_1 and its receptor expression were assessed in a semiquantitative analysis using a visual analogue scale. A subset was analyzed twice to assess the intra-observer variability (kappa = 0.4; $p < 0.05$; deviation in staining grade ranged from 0 to 0.5). The staining intensity in each of the following cell types was scored in a blinded manner: epithelial cells, subepithelial cells ($CD3^+$ and $tryptase^+$ cells, fibroblasts), smooth muscle cells, macrophages ($CD68^+$), and endothelial cells of larger blood vessels. The staining intensity was graded and expressed as: 0 = absence of staining; 1 = moderate staining; 2 = intense staining; 3 = very intense staining.

In Situ Hybridization

The *in situ* hybridization was performed on paraffin-embedded sections adjacent to sections on which TGF-β_1, TGF-βRI, TGF-βRII, and cell type–specific immunoreactivity were assessed. For *in situ* hybridization, we used a *Sma*I-*Bam*HI fragment of TGF-β_1 complementary DNA (cDNA) cloned into pBluescript KS (Stratagene, La Jolla, CA) as described (20). The specific copy RNA (cRNA) probes were labeled with digoxigenin following the manufacturer's protocol (Boehringer, Mannheim, Germany). The *in situ* hybridization was performed essentially as described (20). Briefly, after pretreatment the sections were hybridized with 50 ng per slide during 16 h at 42° C. Subsequently, sections were washed in 2× standard saline citrate (SSC) with 50% formamide at 37° C, then in 0.1× SSC with 20 mM β-mercaptoethanol at 42° C, and finally treated with 2 U/ml ribonuclease (RNAse) T1 (Boehringer, Mannheim, Germany) in 2× SSC plus 1 mM ethylenediaminetetraacetic acid (EDTA) at 37° C. The immunodetection of digoxigenin-labeled hybrids was done using nitro blue tetrazolium (NBT) as chromogen and bicholylindolyl phosphate (BCIP) as coupling agent (Boehringer, Mannheim, Germany). The sense riboprobes were included as negative controls, and in general did not show staining. If staining of sense riboprobes was detected (which was

always far less than the staining of antisense probes), we subtracted this immunostaining score from the equivalent antisense immunostaining score. The staining intensity was expressed as described for the immunocytochemistry.

Statistics

The immunohistochemistry and *in situ* hybridization data were expressed as mean ± SEM. Significance levels were obtained using the unpaired, two-tailed Student's *t* test. Correlation analysis and statistics between expression levels and intraepithelial numbers of mast cells and macrophages was done using Stata Statistical Software 5.0 (StataCorp., College Station, TX). At $p < 0.05$ differences were considered to be statistically significant.

RESULTS

In subjects without COPD, TGF-β_1 mRNA and protein are localized predominantly in bronchial, bronchiolar, and alveolar epithelial cells, vascular and airway smooth muscle cells, and in $CD68^+$ cells (Figures 1 and 2; Table 2). A faint staining was seen in subepithelial cells including inflammatory cells. Although high levels of TGF-β_1 transcripts were found in endothelial cells (Figure 2; Table 2), we did not detect TGF-β_1 immunoreactivity within endothelial cells.

In subjects with COPD, we observed significantly higher ($p < 0.02$) TGF-β_1 protein (up to 2 times) and mRNA (1.5 times) levels in bronchial, bronchiolar, and alveolar epithelial

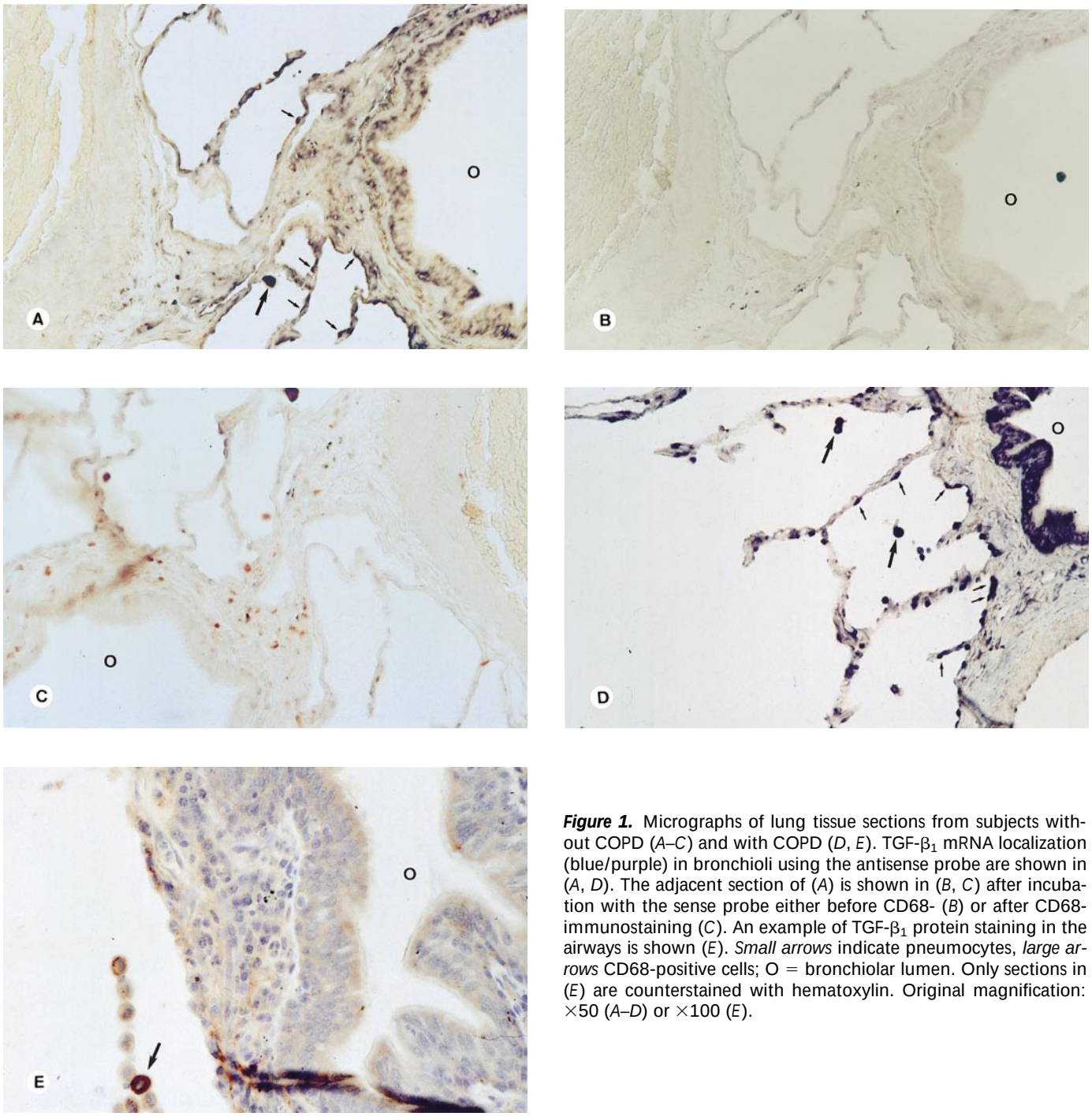

Figure 1. Micrographs of lung tissue sections from subjects without COPD (*A–C*) and with COPD (*D*, *E*). TGF-β_1 mRNA localization (blue/purple) in bronchioli using the antisense probe are shown in (*A*, *D*). The adjacent section of (*A*) is shown in (*B*, *C*) after incubation with the sense probe either before CD68- (*B*) or after CD68-immunostaining (*C*). An example of TGF-β_1 protein staining in the airways is shown (*E*). *Small arrows* indicate pneumocytes, *large arrows* CD68-positive cells; O = bronchiolar lumen. Only sections in (*E*) are counterstained with hematoxylin. Original magnification: ×50 (*A–D*) or ×100 (*E*).

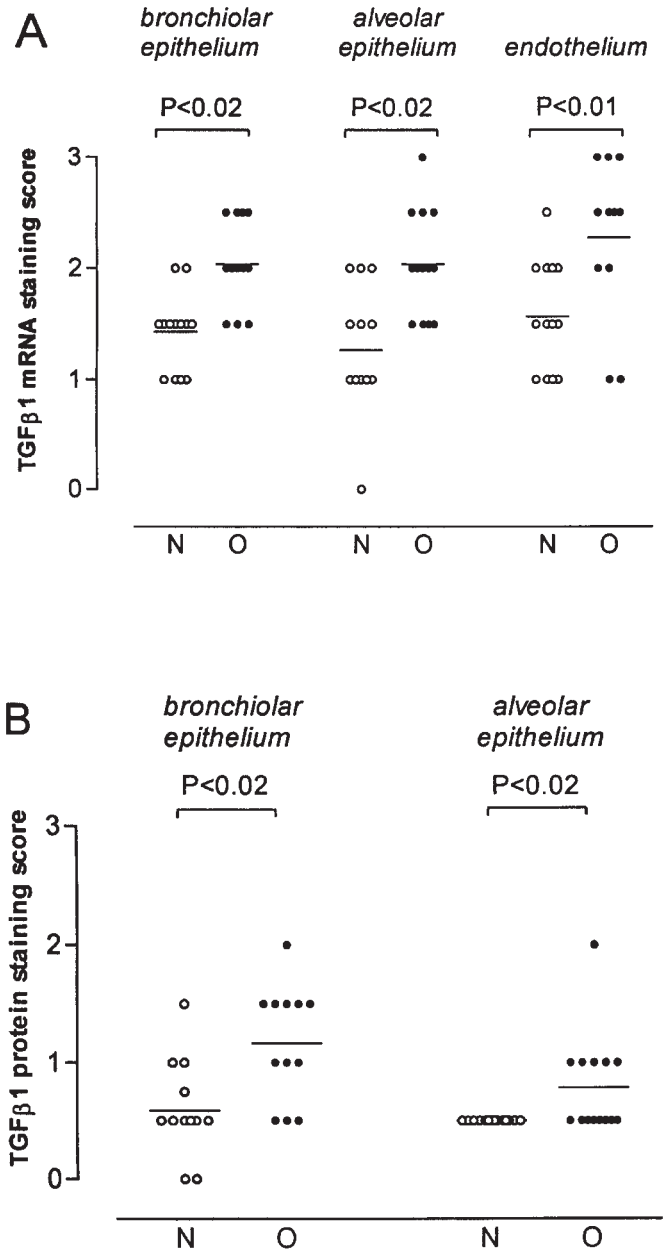

Figure 2. Semiquantitative analysis of the TGF-β_1 mRNA levels (*A*) and TGF-β_1 protein levels (*B*) in bronchiolar epithelium, alveolar epithelium, or endothelial cells within the airway walls. The immunostaining score is given on the *y*-axis ranging from 0 (no staining) to 3 (very intense staining). *Open* and *closed circles* represent individual data from subjects without COPD (N) and with COPD (O), respectively. Means (*horizontal lines*) and significance levels (p values) are indicated.

cells as compared with subjects without COPD (Figure 2; Table 2). In vascular smooth muscle cells within alveoli of subjects with COPD, we also noted 1.5 times higher ($p < 0.002$) TGF-β_1 protein but not mRNA levels (Table 2). Endothelial cells in airway walls but not in alveolar walls exhibit 1.5 times higher ($p < 0.01$) TGF-β_1 mRNA levels as compared with patients without COPD (Figure 2; Table 2). No differences in expression levels of TGF-β_1 were noticed in $CD68^+$.

Because a difference in TGF-βR levels may also contribute to TGF-β_1-mediated effects that underlie the pathogenesis of COPD, we examined both TGF-βRI and TGF-βRII protein

TABLE 2

EXPRESSION OF TGF-β_1 IN PERIPHERAL LUNG TISSUE*

	Airways		Alveoli	
	Non-COPD	COPD	Non-COPD	COPD
Epithelium				
mRNA	1.4 ± 0.1	2.0 ± 0.1†	1.3 ± 0.3	2.0 ± 0.2†
Protein	0.6 ± 0.1	1.2 ± 0.1†	0.5 ± 0.0	0.8 ± 0.1†
Stroma				
mRNA	1.0 ± 0.3	1.2 ± 0.8	0.7 ± 0.1	0.8 ± 0.4
Protein	0.14 ± 0.06	0.04 ± 0.04	0.04 ± 0.04	0.11 ± 0.08
Vascular SMC				
mRNA	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	0.7 ± 0.2
Protein	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.1†
Airway SMC				
mRNA	0.6 ± 0.3	1.1 ± 0.3	1.2 ± 0.1	0.9 ± 0.1
Protein	0.9 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.5 ± 0.0
Endothelium				
mRNA	1.6 ± 0.2	2.3 ± 0.4†	1.6 ± 0.5	1.5 ± 0.3
Protein	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Macrophages				
mRNA	2.1 ± 0.6	2.4 ± 0.2	1.7 ± 0.6	1.9 ± 0.4
Protein	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.2

Definition of abbreviation: SMC = smooth muscle cells.

* Semiquantitative analysis of the TGF-β_1 mRNA and protein levels per cell type in bronchial and bronchiolar airways (airways) or in the alveoli (alveoli) in subjects without (non-COPD) or with COPD. The mean immunostaining score ± SEM ranges from 0 (no staining) to 3 (very intense staining).

† Significant difference ($p < 0.02$, Student's *t* test) with comparable data from subjects without COPD.

expression levels. In subjects without COPD, both receptors are present on the same cells that produce TGF-β_1. The highest expression levels of both receptors are found in bronchial, bronchiolar, and alveolar epithelium as well as in alveolar macrophages (Table 3). Subepithelial cells (fibroblasts, inflammatory cells) exhibited less expression of TGF-βRI and TGF-βRII as compared with epithelial, smooth muscle, or endothelial cells (Table 3). In contrast, endothelial cells display a moderate expression of TGF-βRI and low levels of TGF-βRII (Table 3).

TABLE 3

EXPRESSION OF TGF-β_1 RECEPTORS IN PERIPHERAL LUNG TISSUE*

	Airways		Alveoli	
	Non-COPD	COPD	Non-COPD	COPD
Epithelium				
TGF-βRI	1.6 ± 0.1	1.9 ± 0.2	2.1 ± 0.2	1.9 ± 0.2
TGF-βRII	1.1 ± 0.1	1.3 ± 0.2	1.5 ± 0.1	1.4 ± 0.2
Stroma				
TGF-βRI	0.6 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	1.0 ± 0.1
TGF-βRII	0.1 ± 0.1	0.3 ± 0.1	0.9 ± 0.2	0.9 ± 0.2
Vascular SMC				
TGF-βRI	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
TGF-βRII	0.4 ± 0.1	0.7 ± 0.1	0.50 ± 0.05	0.5 ± 0.1
Airway SMC				
TGF-βRI	1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
TGF-βRII	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.2	0.5 ± 0
Endothelium				
TGF-βRI	1.4 ± 0.1	1.6 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
TGF-βRII	0.3 ± 0.2	0.3 ± 0.2	0 ± 0	0.1 ± 0.1
Macrophages				
TGF-βRI	1.8 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.8 ± 0.1
TGF-βRII	1.7 ± 0.1	1.9 ± 0.2	1.1 ± 0.1	2.0 ± 0.1†

Definition of abbreviation: SMC = smooth muscle cells.

* Semiquantitative analysis of TGF-βRI and TGF-βRII protein levels per cell type in bronchial and bronchiolar airways (airways) or in the alveoli (alveoli) in subjects without (non-COPD) or with COPD. The mean immunostaining score ± SEM ranges from 0 (no staining) to 3 (very intense staining).

† Significant difference ($p < 0.001$, Student's *t* test) with comparable data from non-COPD subjects.

In subjects with COPD, we did not detect expression levels and patterns of TGF-βRI different from those in subjects without COPD. However, a twofold increase ($p < 0.001$) in TGF-βRII expression in alveolar macrophages was observed as compared with subjects without COPD (Table 3).

We found a positive and significant correlation between TGF-β_1 mRNA and protein levels in bronchiolar epithelial cells ($r = 0.62$; $p < 0.002$). In addition, the bronchiolar endothelial TGF-β_1 mRNA levels correlated well with the bronchiolar TGF-β_1 protein levels in vascular smooth muscle cells ($r = 0.58$; $p < 0.006$). In the bronchiolar epithelium, the numbers of intraepithelial $CD68^+$ cells were correlated with both TGF-β_1 mRNA ($r = 0.44$; $p < 0.03$) and protein ($r = 0.44$; $p < 0.03$) levels (Figure 3). The number of intraepithelial $tryptase^+$ cells (considered to be mast cells) also correlated with the TGF-β_1 mRNA levels in the bronchiolar epithelium ($r = 0.58$; $p < 0.002$), but did not correlate significantly with TGF-β_1 protein levels ($r = 0.13$; $p = 0.48$) (Figure 3). Finally, if considering all subjects with and without COPD together, then the FEV_1 values correlate with both TGF-β_1 mRNA ($r = -0.67$; $p < 0.0002$) and protein ($r = -0.60$; $p < 0.002$) expression in the bronchiolar epithelium.

DISCUSSION

In the present study, we compared protein and mRNA distribution patterns of TGF-β_1, and protein expression of TGF-βRI and TGF-βRII in the pulmonary bronchi, bronchioli, and alveoli of subjects with and without COPD. In subjects without COPD, TGF-β_1 proteins and transcripts were seen predominantly in epithelial cells, smooth muscle cells, and both interstitial and intraluminal $CD68^+$ cells (considered herein to be macrophages). The distribution patterns were similar for all airways examined. In subjects with COPD, a higher expression of TGF-β_1 mRNA and protein was seen in airway and alveolar epithelial cells as compared with subjects without COPD. A high expression of both TGF-β receptor types was seen especially in macrophages in subjects with COPD. The higher expression of TGF-β_1 in bronchiolar epithelial cells correlates with both the increased number of macrophages and mast cells in the bronchiolar epithelium in COPD (6), and with FEV_1 values if all current or ex-smokers were taken together. These data indicate that TGF-β_1 is implicated in the recruitment of macrophages and mast cells into the airway epithelium in COPD.

In vitro studies demonstrated that TGF-β mediates chemotaxis of different types of inflammatory cells including monocytes, mast cells, and T lymphocytes (9–12). TGF-β_1 can also stimulate the expression of the cell–cell adhesion molecule, intercellular adhesion molecule-1 (ICAM-1) on endothelial cells (22). ICAM-1 is necessary for diapedesis of mononuclear phagocytes through the endothelial and epithelial cell layers (23). The enhanced ICAM-1 expression as seen on airway epithelial cells in smokers with chronic bronchitis (24) may be

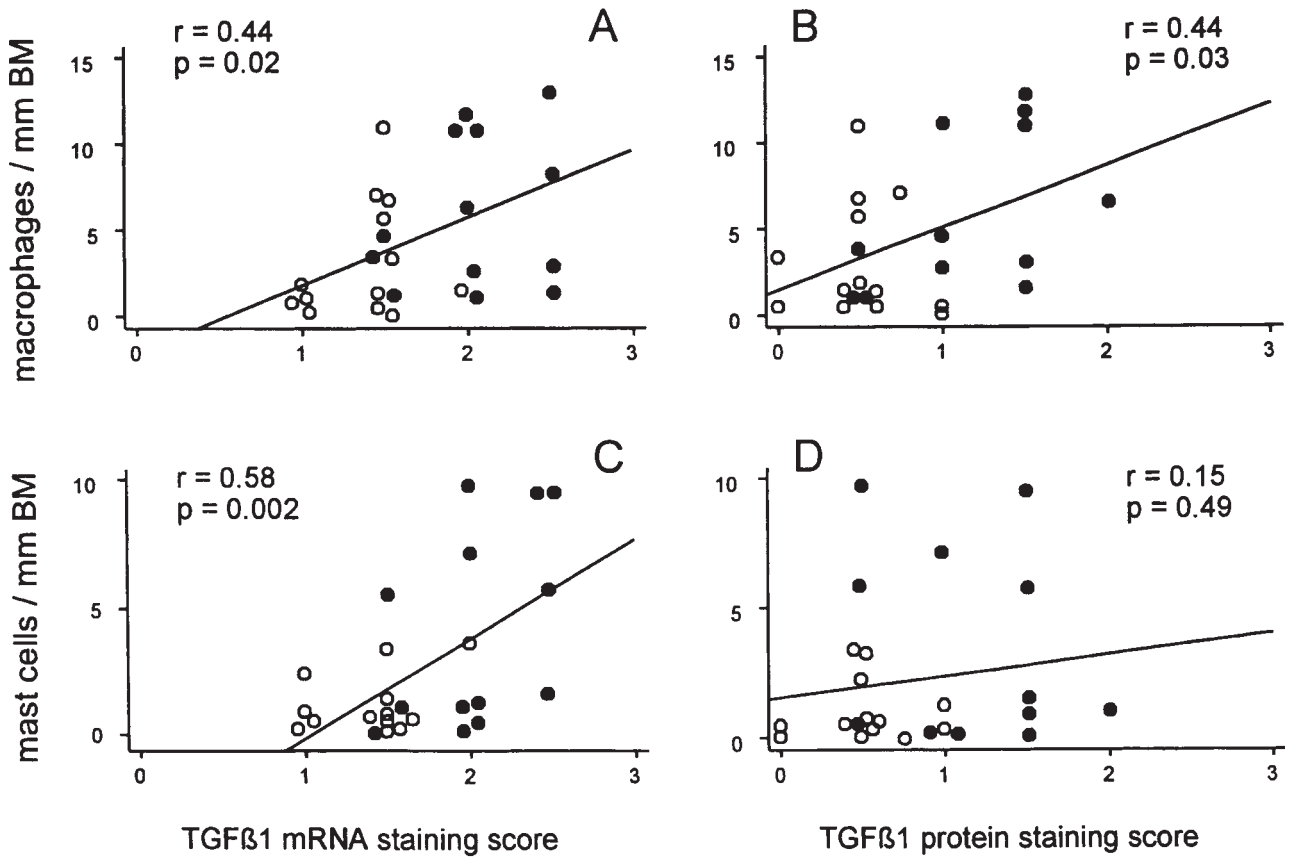

Figure 3. Correlations between inflammatory cell numbers and TGF-β_1 protein and mRNA expression both in bronchiolar epithelium. (*A*) Macrophage numbers and TGF-β_1 mRNA levels. (*B*) Macrophage numbers and TGF-β_1 protein expression. (*C*) Mast cell numbers and TGF-β_1 mRNA levels. (*D*) Mast cell numbers and TGF-β_1 protein expression. Cell numbers are given per millimeter of basement membrane (BM). *Open circles* represent data from subjects without COPD, *closed circles* from subjects with COPD. Data are obtained by linear regression analysis. Correlation (r) and significance level (p value) are given.

mediated by TGF-β_1 and supposedly stimulates the diapedesis of pulmonary monocytes and macrophages. TGF-β_1 can also induce the expression of cytokines like interleukin-1β by monocytes and thereby contributes to inflammatory processes (9, 25). *In vivo* studies on transgenic mice support the *in vitro* data as it was shown that targeted overexpression of TGF-β_1 in the pancreas or in the central nervous system is accompanied by an influx of inflammatory cells including macrophages into these organs (26, 27). Hence, there is ample evidence that TGF-β is involved in inflammation.

Our present expression data on TGF-β_1 in airways of subjects without COPD agree with previous studies on subjects without COPD (13, 14, 19). We also found TGF-β_1 expression in pneumocytes both at the protein and at the mRNA level. In contrast, Aubert and coworkers (18), Magnan and coworkers (13), and Corrin and coworkers (15) did not observe any TGF-β_1 protein immunoreactivity in morphologically normal-appearing pneumocytes. This may be due to the recognition of different epitopes by the anti-TGF-β_1 antibodies. Furthermore, the TGF-β_1 expression patterns in patients with COPD as observed in the present study agree with the two TGF-β_1 expression studies done so far on three patients with emphysema (17) and 19 smokers with chronic bronchitis (19). In contrast, Aubert and coworkers (18) did not find any difference in TGF-β_1 mRNA or protein expression between subjects with or without COPD. A possible explanation could be that Aubert and coworkers (18) did not analyze the TGF-β_1 expression per cell type but rather used tissue homogenates. In addition, as pointed out earlier, the TGF-β_1 protein localization was examined with different antibodies.

Vignola and coworkers (19) reported a higher epithelial TGF-β_1 expression in lung tissue from smokers with chronic bronchitis as compared with nonsmokers. They suggested that cigarette smoking accounts for the higher TGF-β_1 expression. However, they did not include a subject group of smokers without chronic bronchitis. Now, we demonstrate that the epithelial TGF-β_1 expression is increased in smokers with COPD as compared with smokers without COPD. Because the mean number of pack-years does not differ between our two subject groups (Table 1), our data indicate that smoking alone cannot fully account for the higher epithelial TGF-β_1 expression in COPD. In addition, the higher epithelial TGF-β_1 mRNA and protein expression in COPD correlates with a low FEV_1. From our data, we cannot conclude that the TGF-β_1 expression correlates with the severity of the disease. This is probably due to the lack of sufficient numbers of patients with severe COPD.

According to the linear regression analysis, the number of intraepithelial mast cells correlates significantly with the epithelial TGF-β_1 mRNA but not the protein expression. At this moment, we do not have an explanation for this difference. The presence of TGF-β_1 mRNA but absence of TGF-β_1 protein in endothelial cells (Figure 2), and the good correlation between endothelial TGF-β_1 mRNA levels and vascular smooth muscle cells TGF-β_1 protein levels suggest that endothelial cells synthesize no or little TGF-β_1 protein and/or that TGF-β_1 proteins produced by endothelial cells are rapidly secreted and taken up by the surrounding smooth muscle cells.

The relatively high levels of TGF-βRI and TGF-βRII on macrophages in COPD indicate that TGF-β_1 may potentially stimulate the migration of these macrophages. Moreover, the elevated level of TGF-β_1 in lung epithelial cells in COPD provides a concentration gradient of TGF-β_1 which may mediate an even more prominent effect on macrophage migration as compared with subjects without COPD. Because TGF-β_1 is chemotactic toward monocytes/macrophages, this strengthens the hypothesis that lung epithelial–derived TGF-β_1 is involved in the chemotaxis of macrophages into the airway epithelium in COPD.

TGF-β_1 is probably not the only factor in the recruitment of macrophages and mast cells into the bronchiolar epithelium in COPD. Another potent chemotactic and activating protein for macrophages and murine mast cells is monocyte chemoattractant protein-1 (MCP-1) which can be expressed by airway epithelial cells *in situ* (28–30). TGF-β has been shown to induce the expression of MCP-1 (31, 32). In addition, a recent study showed that intratracheal instillation of MCP-1 in mice resulted in a recruitment of macrophages into the lung interstitium and alveoli and enhanced cigarette smoke–induced emphysema (33). Whether the influx of macrophages and mast cells into the airway epithelium in subjects with COPD is mediated directly by TGF-β_1 or indirectly via MCP-1, remains to be determined.

Finally, TGF-β_1 may be involved in structural remodeling of the extracellular matrix. We found a higher TGF-β expression in airway epithelium and pneumocytes in smokers with COPD as compared with smokers without COPD (this study). Other studies have shown an increased mass of structurally disordered collagen bundles in the alveolar septae of subjects with emphysema (7, 8). In bronchial airways, the increased expression of TGF-β_1 in chronic bronchitis is significantly correlated with the number of fibroblasts and the thickness of the basement membrane (19). Finally, TGF-β_1 can induce extracellular matrix synthesis including collagens and fibronectin in various cell types including lung fibroblasts, macrophages, and epithelial cells *in vitro* (34, 35). These data support the hypothesis that TGF-β_1 contributes to the airway and airspace remodeling in COPD.

In conclusion, in smokers without COPD TGF-β_1 and its receptors are expressed differentially by airway epithelial, endothelial, and smooth muscle cells, as well as macrophages. In smokers or ex-smokers with COPD, the higher TGF-β_1 expression in bronchiolar epithelial cells correlated with the increased numbers of intraepithelial macrophages in COPD. The present data strengthen the hypothesis that TGF-β_1 is involved in macrophage influx in COPD. Although the correlation data suggest that TGF-β_1 is not the only factor for this recruitment, our data may point to the importance of TGF-β_1 antagonists in future strategies for therapy of COPD.

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References

1. Jeffery, P. K. 1991. Morphology of the airway wall in asthma and in chronic obstructive pulmonary disease. *Am. Rev. Respir. Dis.* 143: 1152–1158.
2. Fournier, M., F. Lebargy, F. Le Roy Ladurie, E. Lenormand, and R. Pariente. 1989. Intra-epithelial T-lymphocyte subsets in the airways of normal subjects and of patients with chronic bronchitis. *Am. Rev. Respir. Dis.* 140:737–742.
3. Saetta, M., A. Di Stefano, P. Meastrelli, A. Ferraresso, R. Drigo, A. Potena, A. Ciacca, and L. M. Fabbri. 1993. Activated T-lymphocytes and macrophages in bronchial mucosa in subjects with chronic bronchitis. *Am. Rev. Respir. Dis.* 147:301–306.
4. Pesci, A., G. A. Rossi, G. Bertorelli, A. Aufiero, P. Zanon, and D. Olivieri. 1994. Mast cells in the airway lumen and bronchial mucosa of patients with chronic bronchitis. *Am. J. Respir. Crit. Care Med.* 149: 1311–1316.
5. O'Shaughnessy, T. C., T. W. Ansari, N. C. Barnes, and P. K. Jeffery. 1997. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of $CD8^+$ T lymphocytes with FEV_1. *Am. J. Respir. Crit. Care Med.* 155:852–857.
6. Grashoff, W. F. H., J. K. Sont, P. J. Sterk, P. S. Hiemstra, W. I. De Boer, J. Stolk, and J. H. J. M. Van Krieken. 1997. Chronic obstructive pulmonary disease: the role of bronchiolar mast cells and macrophages. *Am. J. Pathol.* 151:1785–1790.

7. Finlay, G. A., M. D. O'Donnell, C. M. O'Connor, J. P. Hayes, and M. X. FitzGerald. 1996. Elastin and collagen remodelling in emphysema: a scanning electron microscopy study. *Am. J. Pathol.* 149:1405–1415.
8. Lang, M. R., G. W. Fiaux, M. Gillooly, J. A. Stewart, D. J. S. Hulmes, and D. Lamb. 1994. Collagen content of alveolar wall tissue in emphysematous and non-emphysematous lungs. *Thorax* 49:319–326.
9. Wahl, S. M., D. A. Hunt, L. M. Wakefield, N. McCartney-Francis, L. M. Wahl, A. B. Roberts, and M. B. Sporn. 1987. Transforming growth factor type β induces monocyte chemotaxis and growth factor production. *Proc. Natl. Acad. Sci. U.S.A.* 84:5788–5792.
10. Allen, J. B., C. L. Manthey, A. R. Hand, K. Ohura, L. Ellingsworth, and S. M. Wahl. 1990. Rapid onset of synovial inflammation and hyperplasia induced by transforming growth factor β. *J. Exp. Med.* 171:231–247.
11. Adams, D. H., M. Hathaway, J. Shaw, D. Burnett, E. Elias, and A. J. Strain. 1991. Transforming growth factor-β induces human T lymphocyte migration in vitro. *J. Immunol.* 147:609–612.
12. Gruber, B. L., M. J. Marchese, and R. R. Kew. 1994. Transforming growth factor-β1 mediates mast cell chemotaxis. *J. Immunol.* 152: 5860–5867.
13. Magnan, A., I. Frachon, B. Rain, M. Peuchmauer, G. Monti, B. Lenot, M. Fattal, G. Simonneau, P. Galanaud, and D. Emilie. 1994. Transforming growth factor β in normal human lung: preferential location in bronchial epithelial cells. *Thorax* 49:789–792.
14. Coker, R. K., G. J. Laurent, S. Shahzeidi, N. A. Hernandez-Rodriguez, P. Pantelides, R. M. Du Bois, P. K. Jeffery, and R. J. McAnulty. 1996. Diverse cellular TGF-β1 and TGF-β3 gene expression in normal human and murine lung. *Eur. Respir. J.* 9:2501–2507.
15. Corrin, B., D. Butcher, B. J. McAnulty, R. M. Dubois, C. M. Black, G. J. Laurent, and N. K. Harrison. 1994. Immunohistochemical localization of transforming growth factor-β1 in the lungs of patients with systemic sclerosis, cryptogenic fibrosing alveolitis and other lung disorders. *Histopathology* 24:145–150.
16. Jagirdar, J., R. Begin, A. Dufresne, S. Goswami, T. C. Lee, and W. N. Rom. 1996. Transforming growth factor-β (TGF-β) in silicosis. *Am. J. Respir. Crit. Care Med.* 154:1076–1081.
17. Khalil, N., R. N. O'Connor, H. W. Unruh, P. W. Warren, K. C. Flanders, A. Kemp, O. H. Bereznay, and A. H. Greenberg. 1991. Increased production and immunohistochemical localization of transforming growth factor-β in idiopathic pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 5:155–162.
18. Aubert, J.-D., B. I. Dalai, T. R. Bai, C. R. Roberts, S. Hayashi, and J. C. Hogg. 1994. Transforming growth factor β1 gene expression in human airways. *Thorax* 49:225–232.
19. Vignola, A. M., P. Chanez, G. Chiappara, A. Merendino, E. Pace, A. Rizzo, A. M. La Rocca, V. Bellia, G. Bonsignore, and J. Bousquet. 1997. Transforming growth factor-β expression in mucosal biopsies in asthma and chronic bronchitis. *Am. J. Respir. Crit. Care Med.* 156: 591–599.
20. De Boer, W. I., A. G. P. Schuller, M. Vermey, and T. H. Van der Kwast. 1994. Expression of growth factors and receptors during specific phases in regenerating urothelium after acute injury in vivo. *Am. J. Pathol.* 145:1199–1207.
21. De Boer, W. I., M. Vermeij, S. Gil Diez de Medina, E. Bindels, F. Radvanyi, T. Van der Kwast, and D. Chopin. 1996. Functions of fibroblast and transforming growth factors in primary organoid-like cultures of normal human urothelium. *Lab. Invest.* 75:147–156.
22. Suzuki, Y., T. Tanigaki, D. Heimer, W. Wang, W. G. Ross, G. A. Murphy, A. Sakai, H. H. Sussman, T. H. Vu, and T. A. Raffin. 1994. TGF-beta 1 causes increased endothelial ICAM-1 expression and lung injury. *J. Appl. Physiol.* 77:1281–1287.
23. Albelda, S. M. 1991. Endothelial and epithelial cell adhesion molecules. *Am. J. Respir. Cell Mol. Biol.* 4:195–203.
24. Di Stefano, A., P. Maestrelli, A. Roggeri, G. Turato, S. Calabro, A. Potena, C. E. Mapp, A. Ciaccia, L. Covacev, L. M. Fabbri, and M. Saetta. 1994. Upregulation of adhesion molecules in the bronchial mucosa of subjects with chronic obstructive bronchitis. *Am. J. Respir. Crit. Care Med.* 149:803–810.
25. Bombara, C., and R. A. Ignotz. 1992. TGF-beta inhibits proliferation of and promotes differentiation of human promonocytic leukemia cells. *J. Cell. Physiol.* 153:30–37.
26. Lee, M. S., D. Gu, L. Feng, S. Curriden, M. Arnush, T. Krahl, D. Gurushanthaiah, C. Wilson, D. L. Loskutoff, H. Fox, and N. Sarvetnick. 1995. Accumulation of extracellular matrix and developmental dysregulation in the pancreas by transgenic production of transforming growth factor-beta 1. *Am. J. Pathol.* 147:42–52.
27. Wyss-Coray, T., P. Borrow, M. J. Brooker, and L. Mucke. 1997. Astroglial overproduction of TGF-beta 1 enhances inflammatory central nervous system disease in transgenic mice. *J. Neuroimmunol.* 77:45–50.
28. Conti, P., W. Boucher, R. Letourneau, C. Feliciano, M. Reale, R. C. Barbacane, P. Vlagopoulos, G. Bruneau, J. Thibault, and T. C. Theoharides. 1995. Monocyte chemotactic protein-1 provokes mast cell aggregation and [3H]5HT release. *Immunology* 86:434–440.
29. Antoniades, H. N., J. Neville-Golden, T. Galanopoulos, R. L. Kradin, A. J. Valente, and D. T. Graves. 1992. Expression of monocyte chemoattractant protein 1 mRNA in human idiopathic pulmonary fibrosis. *Proc. Natl. Acad. Sci. U.S.A.* 89:5371–5375.
30. De Boer, W. I., W. A. A. M. Van Schadewijk, J. Stolk, J. H. J. M. Van Krieken, and P. S. Hiemstra. 1997. Increased pulmonary expression of transforming growth factor β_1 (TGFβ_1) and monocyte chemoattractant protein 1 (MCP-1) in chronic obstructive pulmonary disease (abstract). *In* Chemokines in Disease Models and Pathogenesis. NMHCC Second International Conference. National, Managed Health Care Congress, Waltham.
31. Villiger, P. M., R. Terkeltaub, and M. Lotz. 1992. Production of monocyte chemoattractant protein-1 by inflamed synovial tissue and cultured synoviocytes. *J. Immunol.* 149:722–727.
32. Slavin, J., E. Unemori, T. K. Hunt, and E. Amento. 1995. Monocyte chemotactic protein-1 (MCP-1) mRNA is down-regulated in human dermal fibroblasts by dexamethasone: differential regulation by TGF-beta. *Growth Factors* 12:151–157.
33. Hautamaki, R. D., D. K. Kobayashi, R. M. Senior, and S. D. Shapiro. 1997. Requirement for macrophage elastase for cigarette smoke–induced emphysema in mice. *Science* 277:2002–2004.
34. Rennard, S. I., G. W. Hunninghake, P. B. Bitterman, and R. G. Crystal. 1981. 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. U.S.A.* 78:7147–7151.
35. Kawamoto, M., D. J. Romberger, Y. Nakamura, Y. Adachi, L. Tate, R. F. Ertl, J. R. Spurzem, and S. I. Rennard. 1995. Modulation of fibroblast type I collagen and fibronectin production by bovine bronchial epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 12:425–433.