Enhanced Bronchial Expression of Extracellular Matrix Proteins in Chronic Obstructive Pulmonary Disease

Andor R. Kranenburg, PhD,[1] Anna Willems-Widyastuti, MSc,[1] Wolter J. Mooi, MD, PhD,[2] Peter J. Sterk, MD, PhD,[3] Vijay K.T. Alagappan, MBBS,[1] Willem I. de Boer, PhD,[3] and Hari S. Sharma, PhD, DSc[1]

Key Words: Chronic obstructive pulmonary disease; Airway remodeling; Extracellular matrix; Fibrosis; Human

DOI: 10.1309/JC477FAEL1YKV54W

Abstract

*Remodeling of airways and blood vessels is an important feature in chronic obstructive pulmonary disease (COPD). By using immunohistochemical analysis, we examined bronchial expression patterns of various extracellular matrix (ECM) components such as collagens (subtypes I, III, and IV), fibronectin, and laminin β2 in patients with COPD (forced expiratory volume in 1 second [FEV_1] ≤ 75%; n = 15) and without COPD (FEV_1 ≥85%; n = 16) and correlated expression data with lung function. Quantitative analysis revealed enhanced levels (*P < .01*) of total collagens I, III, and IV in surface epithelial basement membrane (SEBM) and collagens I and III in bronchial lamina propria (*P < .02*) and adventitia (*P < .05*) in COPD. Distinct and increased (*P < .05*) vascular expression of fibronectin accounts for intimal vascular fibrosis, whereas laminin β2 (*P < .05*) was elevated in airway smooth muscle (ASM). FEV_1 values inversely correlated with collagens in the SEBM, fibronectin in bronchial vessels, and laminin in the ASM. Our data suggest that COPD exhibits increased bronchial deposition of ECM proteins that contribute to deteriorated lung function and airway remodeling.*

With increasing morbidity and mortality, chronic obstructive pulmonary disease (COPD) remains a global health problem. One of the major causal factors is tobacco smoking, but of all smokers, COPD develops in only 10% to 20%.[1] Pathologic features of COPD include thickening of airway walls, probably as a result of ongoing chronic inflammatory processes with an influx of neutrophils, macrophages, and T lymphocytes.[2,3] The resultant changes in the airway wall in COPD include hyperplasia of subepithelial myofibroblasts and airway smooth muscle (ASM) cells.[2,3]

Previous studies on the pathology of COPD have focused on alterations in small airways and parenchyma, where an infiltration of CD8+ T cells and macrophages, a loss in the number of alveolar-bronchiolar attachments and emphysema with consequent loss of elastic recoil, and alveolar-peribronchial wall fibrosis with increased deposition of extracellular matrix (ECM) proteins have been demonstrated.[3-5] In a recent study, Hogg et al[6] have shown that the progression of COPD is associated with the accumulation of inflammatory mucous exudates in the small airways. Thus far, only a few studies on COPD have focused on the larger airways where bronchial epithelial loss and changes in large airway dimensions have been reported in COPD.[2,7-10] Tiddens and coworkers[7] reported that the thickness of the wall area internal to the ASM was increased in COPD and that this increase correlated inversely with the forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio, but these authors found no significant increase in the ASM mass.

Thickening of the surface epithelial basement membrane (SEBM), subepithelial fibrosis, and the deposition of ECM proteins in the lamina propria are key features in asthma.[11,12] In COPD, however, changes in thickness of the SEBM and

fibrosis of the mucosal lamina propria are less pronounced.[12] Studies have indicated that the SEBM thickness in bronchial biopsy specimens from smokers with chronic bronchitis was similar to that in healthy subjects, unless features of asthma such as hyperresponsiveness or corticosteroid sensitivity were present as well.[9,12,13] However, it has not been studied in detail whether the composition of the SEBM is unchanged in COPD, and, in addition, the lamina propria and adventitia may be altered.

We hypothesized that alteration in total or relative content of ECM proteins such as collagens, including subtypes I, III, and IV, fibronectin, laminins, and proteoglycans in the various compartments of the bronchial wall (SEBM, lamina propria, bronchial adventitia, and smooth muscle) are present in the airways of smokers and ex-smokers with COPD. We studied the quantitative distribution patterns of various ECM markers in bronchial tissue samples from smokers and ex-smokers with or without COPD.

Materials and Methods

Selection of Specimens

Bronchial tissue samples from current and/or ex-smokers, who underwent surgery for lung cancer, were obtained at Leiden University Medical Centre, Leiden, the Netherlands, and Southern Hospital, Rotterdam, the Netherlands, after approval of the study by the Leiden University Medical Centre Medical Ethics Committee. Lung tissue samples were fixed for 24 hours by perfusion fixation using an injection syringe filled with 10% phosphate-buffered formalin, embedded in paraffin, and processed for immunohistochemical analysis. Samples of bronchial airways, located as far away as possible from the tumor, were chosen for the study. Based on lung function outcome, patients were assigned to COPD and non-COPD groups.[14-16] The patients in these 2 groups participated in a larger research project, part of which has been published.[10,16]

COPD Group

Fifteen subjects were assigned to this group on the basis of the following parameters: an FEV_1 of 75% or less of predicted value before bronchodilatation, an FEV_1/FVC ratio less than 75%, a reversibility in FEV_1 of 12% or less of the predicted value after 400 μg of inhaled albuterol (salbutamol), and a transfer factor for carbon monoxide (diffusion capacity) per liter of alveolar volume (K_{co}) of 80% or less of the predicted value.[17]

Non-COPD Group

Sixteen subjects were assigned to this group based on the basis of the following data: an FEV_1 of 85% or more of predicted value before bronchodilatation, an FEV_1/FVC ratio of more than 85%, and reversibility in FEV_1 of 12% or less of the predicted value after 400 μg of albuterol inhalation. To exclude accompanying lung disease leading to a restrictive lung function, it was required that the total lung capacity (TLC) of each subject be more than 80% of the predicted value.[17]

Clinical data for all patients were examined for possible comorbidity and medication use. All patients were free of symptoms of upper respiratory tract infection, and none received antibiotics perioperatively. None of the patients received glucocorticosteroids during the 3 months before operation, but 4 patients received oral glucocorticosteroids perioperatively. After selection based on lung function, all lung tissue samples used for the study were checked histologically by using the following exclusion criteria: (1) presence of tumor, (2) presence of poststenotic pneumonia, (3) fibrosis of lung parenchyma, and (4) obstruction of the main bronchus.[14,15]

Pulmonary Function Tests

All pulmonary function tests were performed within 3 months before surgery. The FEV_1 and FVC were measured by spirometry, TLC and residual volume with the closed circuit helium dilution test, and the K_{co} using the single breath-holding technique, as described by Quanjer et al.[17] Lung function data and other patient characteristics are shown in **Table 1**.

Staining of Total Collagen Fibers

The total collagen fibers in bronchial tissue specimens were stained with Picro-sirius red F3BA (Sigma, St Louis, MO).[18] Tissue sections, 4 μm thick, were treated with 0.2% aqueous phosphomolybdic acid and incubated in 0.1% Picro-sirius red. Before dehydration, the slides were treated with 0.01N hydrochloric acid and mounted. Slides were visualized under a light microscope, and collagen content was assessed using a visual scoring method (see "Quantitative Analysis of Staining").

Immunohistochemical Analysis

Sections of paraffin-embedded lung tissue were cut at 4 μm and mounted on Super Frost Plus microscopic slides (Meinzl-Gläser, Braunschweig, Germany), and serial sections were used to detect the staining of collagen I, III, and IV, fibronectin, and laminin β2 using immunohistochemical analysis. Antihuman mouse monoclonal antibodies against collagen IV, fibronectin, and laminin were purchased from NeoMarkers, Fremont, CA; collagen I from Sigma; and collagen III from BioGenex, San Ramon, CA.

Sections were deparaffinized, rehydrated, and preincubated with 10% normal goat serum diluted in 5% bovine serum albumin in phosphate-buffered saline (PBS), pH 7.4. Subsequently, sections were incubated overnight at 4°C with primary antibodies against collagen I (1:150 vol/vol) or III (undiluted), fibronectin (1:500 vol/vol), and laminin β2 (1:150 vol/vol) or for 1 hour at room temperature for collagen

DOI: 10.1309/JC477FAEL1YKV54W

Table 1
Subject Characteristics and Clinical Parameters*

Case No./Sex	Age (y)	FEV_1	FEV_1/FVC	TLC	RV	Pack-Years	Preoperative Steroid Use
Non-COPD							
1/M	72	98	104	105	122	65	No
2/M	67	102	99	112	134	29	No
3/F	73	91	91	107	125	50	No
4/M	46	109	104	97	87	23	No
5/M	58	96	92	110	121	70	No
6/M	57	94	90	95	87	35	No
7/M	86	86	96	106	140	70	No
8/M	64	93	95	110	152	20	No
9/M	28	99	102	104	143	0	No
10/M	51	97	107	99	92	0	No
11/M	38	100	94	106	100	28	No
12/M	52	100	100	103	121	20	No
13/F	58	100	105	90	90	28	No
14/M	69	110	124	100	102	Unknown	Unknown
15/F	61	94	107	119	142	Unknown	No
16/M	61	86	94	95	119	Unknown	No
Mean ± SEM	58.8 ± 3.5	97 ± 1.6	100 ± 2.1	104 ± 1.9	117 ± 5.4	44 ± 8.6	—
COPD							
17/M	77	73	70	103	110	25	No
18/M	71	69	64	115	129	Unknown	No
19/M	72	37	42	136	229	50	Yes
20/M	60	75	66	123	155	45	No
21/M	53	44	70	89	137	32	No
22/M	65	52	60	112	169	55	No
23/M	55	56	68	99	131	40	No
24/M	55	45	60	Unknown	Unknown	35	Yes
25/M	45	75	74	97	97	Unknown	Unknown
26/M	61	49	62	130	223	20	Yes
27/M	65	69	71	116	152	20	No
28/M	57	47	53	111	170	55	No
29/F	78	62	61	95	105	60	No
30/M	71	45	52	114	171	Unknown	No
31/M	77	67	72	104	128	Unknown	Unknown
Mean ± SEM	64.1 ± 2.6	54 ± 3.3	58 ± 2.3	103 ± 3.6	141 ± 10	31 ± 0.3	—
P	.239	.0001	.0001	.099	.008	.312	—

COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity.
* FEV_1, FVC, TLC, and RV are given as percentage of predicted value. *P* values are given as COPD vs non-COPD.

IV (1:150 vol/vol). Immunostaining was performed after antigen retrieval by 0.1% protease treatment in PBS for 10 minutes at 37°C or, for collagen I, by boiling in citrate buffer (10 mmol/L of citrate buffer, pH 6.0) for 10 minutes in a microwave oven. Incubation for 30 minutes with secondary biotinylated anti-immunoglobulins (Multilink, dilution 1:75; BioGenex) and a tertiary complex of peroxidase-conjugated streptavidin at a dilution of 1:50 were used to enhance the detection sensitivity. Color was developed using 0.025% of 3,3-diaminobenzidine (Sigma, St Louis) in 0.01 mol/L of PBS, containing 0.03% hydrogen peroxide.

Positive control samples consisted of human breast carcinoma and placental tissue. The optimal dilution for all antibodies was identified by examining the intensity of staining obtained with a series of dilutions: the optimum concentration resulted in specific and easily visible signal on paraffin sections of control specimens. Slides were counterstained with Mayer hematoxylin, mounted, and studied light microscopically.

Quantitative Analysis of Staining

A visual scoring method was applied. For this purpose, all tissue samples were analyzed in a blinded manner in random order by 2 independent observers (A.R.K. and A.W.W.) who were unaware of the clinical data for the case under study. Quantitative analysis was performed using a validated, arbitrary visual scale with grading scores of 0, 1, 2, 3, and 4 representing no, weak, moderate, intense, and very intense staining, respectively.[10,14-16,19] We quantified the staining pattern of ECM proteins in the SEBM and subdivided the staining for sites where the bronchial epithelium was totally lost and the SEBM was denuded or not. Furthermore, the interstitial staining of the bronchial lamina propria and adventitia was assessed. Moreover, the staining pattern within the microvasculature bronchial lamina propria or the adventitia was measured. The intensity of laminin expression in the ASM area was quantified. We also examined errors within and between observers by correlating the

DOI: 10.1309/JC477FAEL1YKV54W

expression scores using Pearson analysis and found a very high correlation of 0.8 to 0.9.

Statistical Analysis

Data were analyzed for statistical significance using the unpaired, 2-tailed Student *t* test and the nonparametric Mann-Whitney test, as appropriate. The expression data for ECM proteins were expressed as mean ± SEM. Furthermore, ECM proteins staining for different compartments were correlated with FEV_1 by using Pearson correlation analysis. The individual collagen subtype values were correlated with total collagen staining and with each other to evaluate colocalization. Differences with a *P* value of .05 or less were considered statistically significant.

Results

Clinical Parameters

The clinical and lung function characteristics of all subjects included in the study are listed in Table 1. The COPD group demonstrated an elevated residual volume, whereas the K_{co} was reduced ($P < .005$). Subjects in the 2 groups did not differ significantly in age, TLC, reversibility in FEV_1, smoking status (pack-years), or steroid use (Table 1).

Localization and Quantification of Extracellular Matrix Proteins

We studied the localization of ECM proteins in the bronchial airways **Image 1** and **Image 2**. ECM proteins were assessed systematically in the following sites: the SEBM,

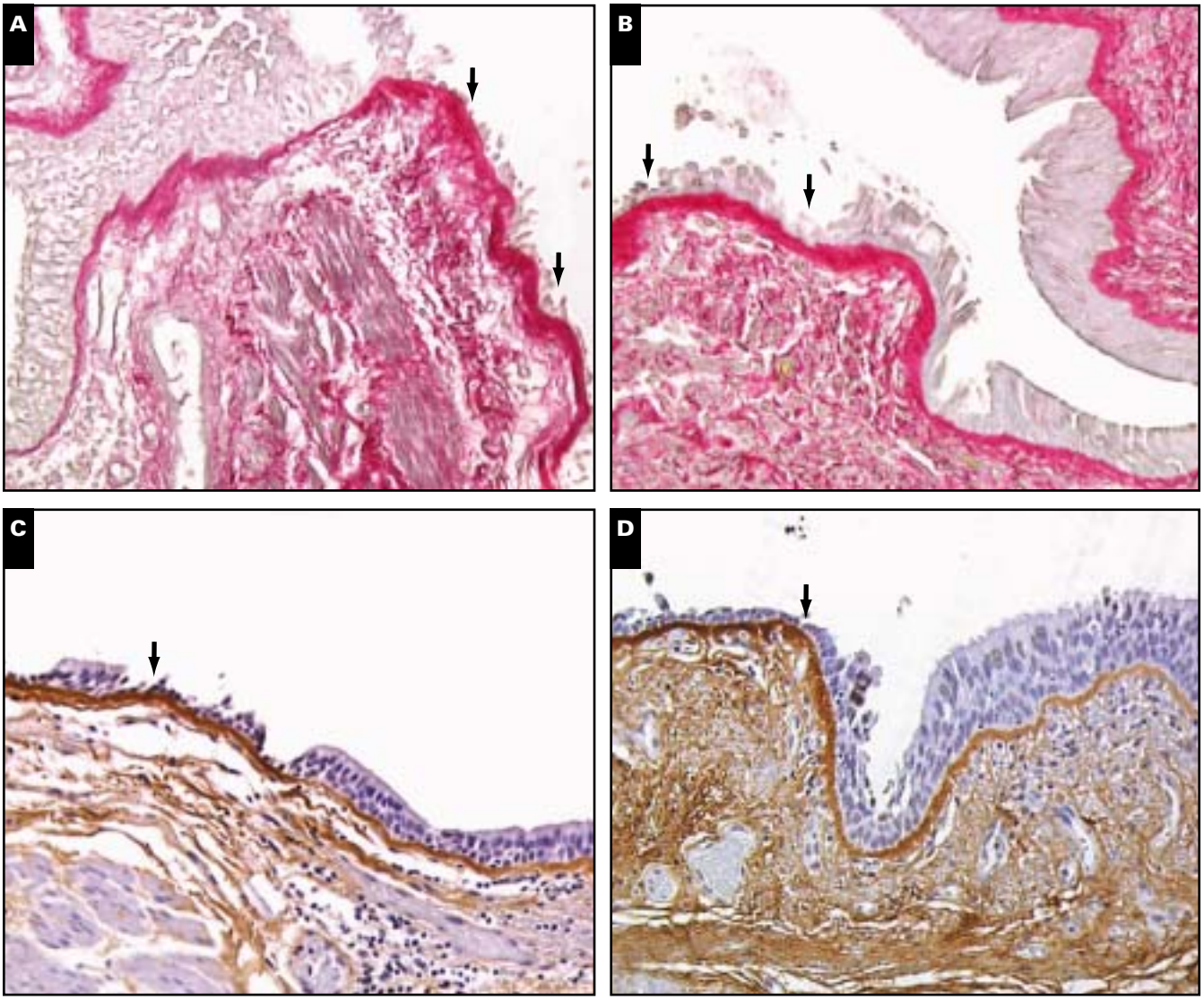
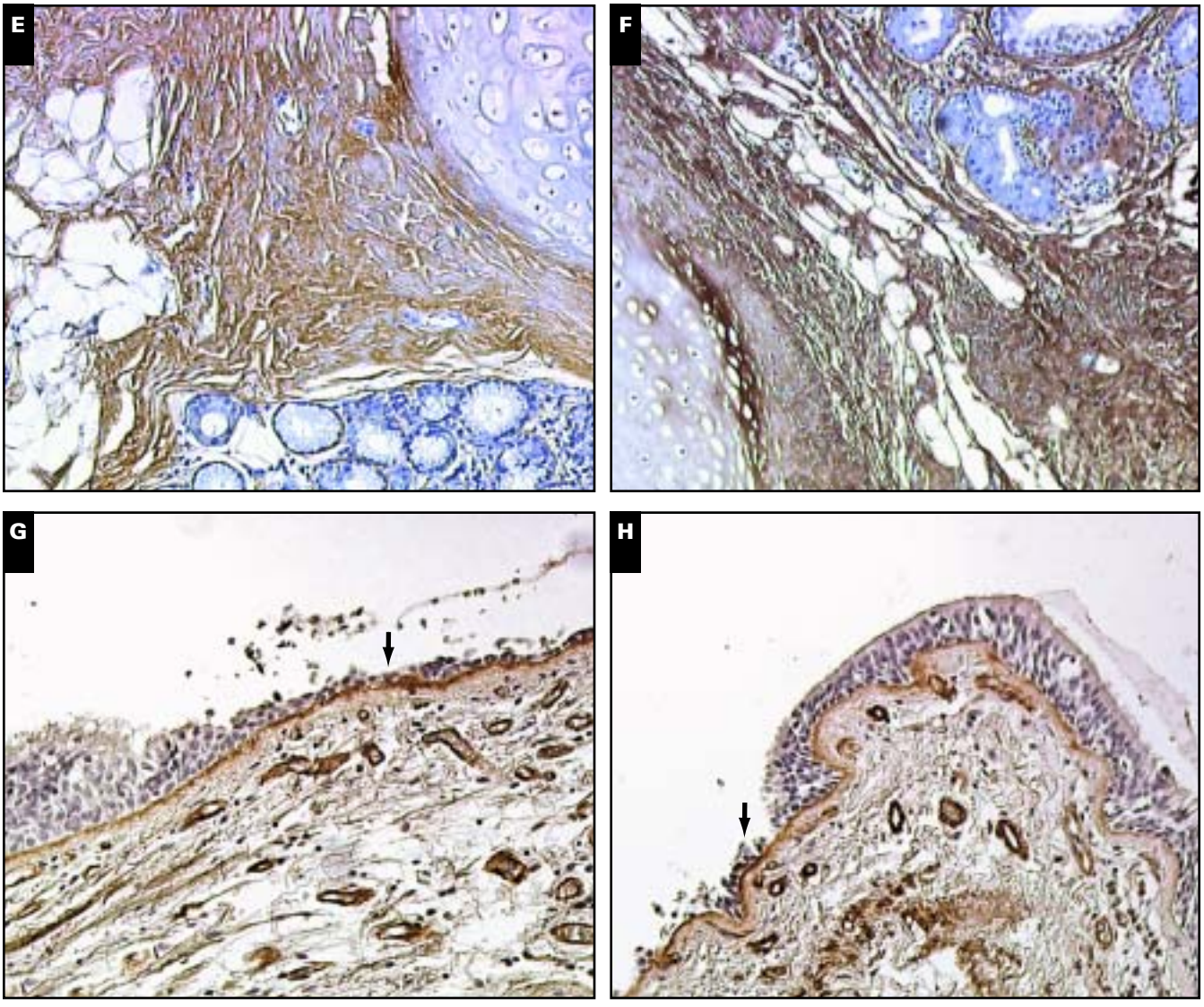


Image 1 Bronchial tissue sections from patients without chronic obstructive pulmonary disease (COPD) (**A**, **C**, **E**, and **G**) and with COPD (**B**, **D**, **F**, and **H**). **A** and **B**, Total collagen staining (sirius-red staining) in bronchial airway walls. **C** and **D**, Staining for collagen I in surface epithelial basement membrane and lamina propria.

DOI: 10.1309/JC477FAEL1YKV54W

connective tissue samples of the lamina propria and adventitia of the bronchial airway, and in the bronchial blood vessels. We observed staining for collagen IV, fibronectin, and laminin within the SEBM relatively more toward the apical side, whereas collagen I and III were localized toward the lamina propria in the reticular layer. Within vessel walls, staining for fibronectin was found in the (neo-)intima, for collagen IV and laminin in the medial layer, and for collagen I and III in the adventitial layer. In addition, laminin was immunolocalized at the apical side of the bronchial epithelium and in the ASM cell layer.

Representative examples of collagen staining in non-COPD (A, C, E, and G) and COPD (B, D, F, and H) samples are depicted in Images 1 and 2. We quantified the staining pattern of ECM proteins in the SEBM and subdivided the staining for sites where the bronchial epithelium was damaged or not. All studied ECM proteins were increased significantly at sites of epithelial denudation **Figure 1** ($P < .01$). We observed more intense staining for total collagen in the SEBM at sites of intact epithelium in subjects with COPD (1.5-fold increase; $P < .05$; Figure 1A). Figure 1B demonstrates that collagen I deposition was increased in COPD compared with that in non-COPD patients in the SEBM at the areas of intact epithelium (2.3-fold increase; $P < .001$) and damaged bronchial epithelium (1.6-fold increase; $P < .01$), and lamina propria and bronchial adventitia (1.9-fold increase each; $P < .001$). Figure 1C indicates that in patients with COPD, collagen III staining is elevated in the SEBM at sites of intact and damaged epithelium (1.5- and 1.4-fold increases, respectively, $P < .01$). Furthermore, at fibrotic sites of lamina propria (1.4-fold



E and **F**, Collagen III protein staining in bronchial adventitial layer with bronchial vessels. **G** and **H**, Collagen IV staining in lamina propria. Arrows indicate sites of damaged bronchial epithelium (**A**-**H**, hematoxylin counterstain, original magnification ×200).

DOI: 10.1309/JC477FAEL1YKV54W

increase; $P < .05$) and adventitia (1.3-fold increase; $P < .05$) of the airway wall, collagen III staining also is increased (Figure 1C). Collagen IV protein, however, remained unaltered, irrespective of the presence of COPD (Figure 1D). Fibronectin deposition was higher in the intima and (neo-)intima, including endothelial cells of bronchial blood vessels in COPD (Figure 1E). Laminin staining was more intense in the ASM layer (1.5-fold; $P < .01$) and small vessels in the lamina propria (1.3-fold; $P < .01$; Figure 1F). No other staining differences were observed between samples from subjects with or without COPD.

Correlation of ECM Proteins With Clinical Data

Pearson correlations of ECM components with FEV_1 values (percentage predicted) in all COPD and non-COPD patients are summarized in **Figure 2**. We observed a significant inverse correlation with FEV_1 of the following parameters: total collagen staining in the SEBM under intact epithelium ($r = –0.47$; $P < .01$); collagen I staining in SEBM at sites with damaged epithelium ($r = –0.61$; $P < .01$); connective tissue of the bronchial adventitia ($r = –0.67$; $P < .001$; Figure 2A) and of the lamina propria ($r = –0.53$; $P < .01$). In the same regions, inverse correlations also were found between collagen III and FEV_1 ($r = –0.40$, $r = –0.42$, and $r = –0.48$; $P < .01$; Figure 2B). Figure 2C illustrates that fibronectin also was correlated inversely with FEV_1 values in endothelium ($r = –0.51$; $P < .01$). Moreover, in ASM, we found a significant inverse correlation between FEV_1 values and laminin ($r = –0.61$; $P < .001$; Figure 2D). When considering colocalization of total collagen with subtypes for collagen I, III, and IV,

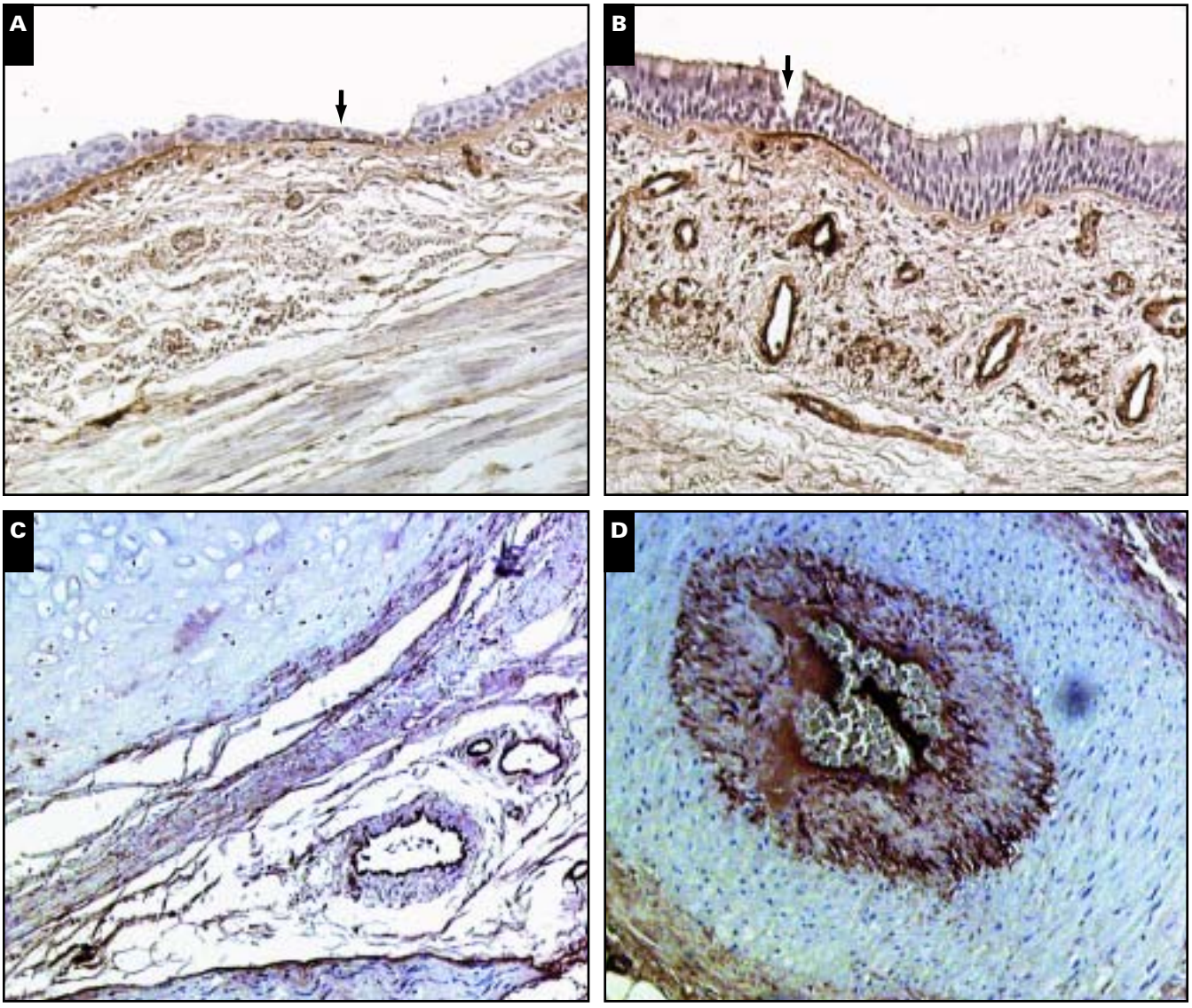


Image 2 Bronchial tissue sections from patients without chronic obstructive pulmonary disease (COPD) (**A**, **C**, **E**, and **G**) and with COPD (**B**, **D**, **F**, and **H**). Fibronectin staining in bronchial lamina propria (**A** and **B**) and vasculature (**C** and **D**).

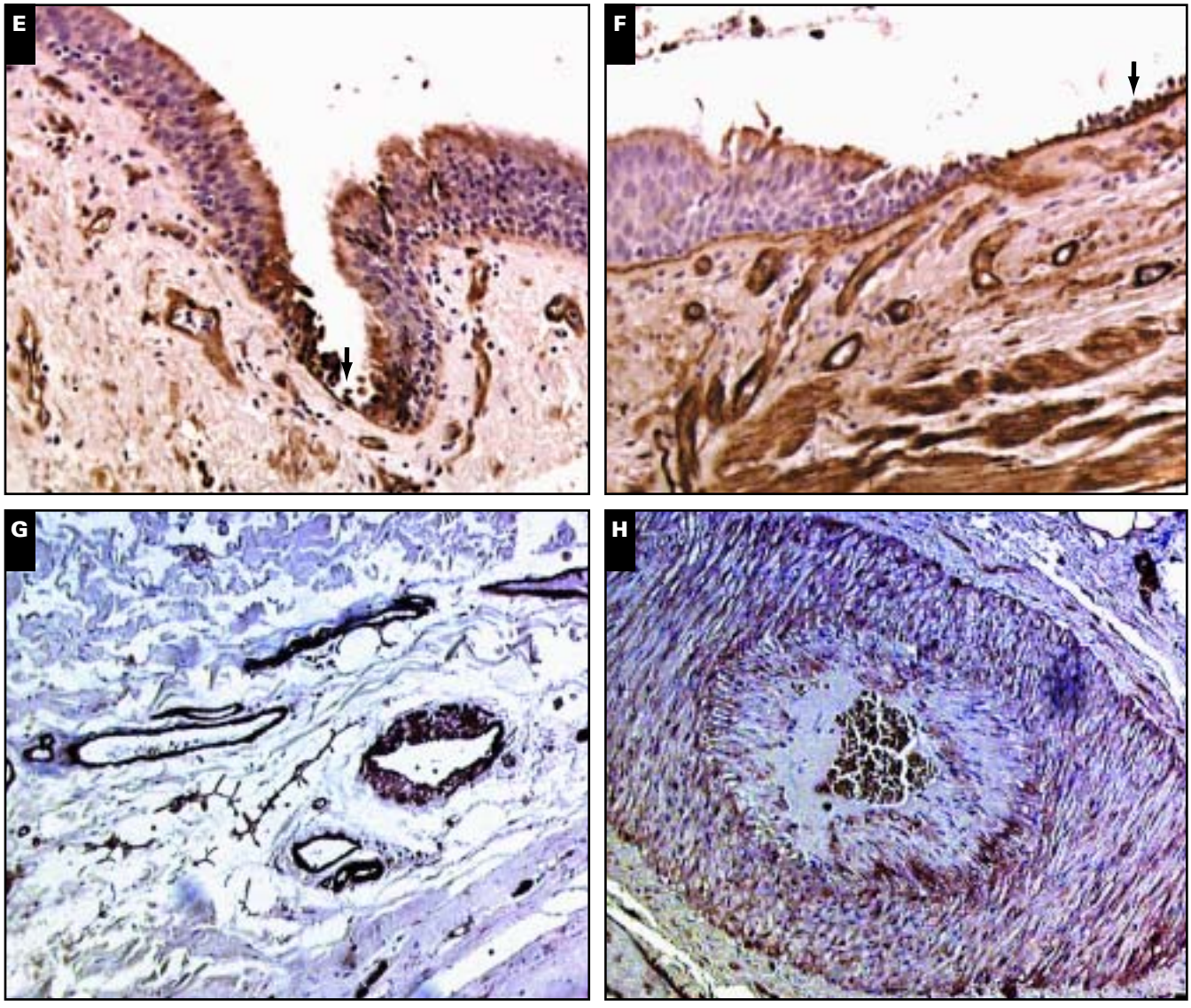
DOI: 10.1309/JC477FAEL1YKV54W

we found a significant correlation between total collagen and collagen III in the SEBM at damaged ($r = 0.62$; $P < .001$) and intact epithelium ($r = 0.63$; $P < .001$). No significant correlation was observed between total collagen and collagen I and IV localization.

Discussion

In this study, we showed that COPD is associated with an increased bronchial deposition of collagens I, III, and IV; fibronectin; and laminin. ECM proteins were observed in SEBM, lamina propria, and adventitia of the bronchial walls and vasculature. We found that ECM protein deposition is increased in the SEBM at sites of damaged bronchial epithelium in all patients. In patients with COPD, total collagen and predominantly collagens I and III were increased further compared with levels in control subjects, whereas bronchial vessels showed increased deposition of fibronectin and laminin. FEV_1 values inversely correlated with collagens in the SEBM, fibronectin in bronchial vessels, and laminin in the ASM. Taken together, these findings suggest that deposition of ECM components contributes to the airway remodeling in COPD.

An identical localization pattern of the various studied ECM markers in the cartilaginous bronchial wall was present in our patient groups, which is in agreement with earlier reports describing their presence in the bronchial airways of patients with asthma and patients with bronchopulmonary dysplasia.[11,20-22] Several reports have demonstrated structural changes with fibrosis and deposition of ECM proteins and loss



Laminin protein staining in the lamina propria (**E** and **F**) and the adventitial layers with bronchial vessels (**G** and **H**). Arrows indicate sites of damaged bronchial epithelium (**A**-**H**, hematoxylin counterstain, original magnification ×200).

DOI: 10.1309/JC477FAEL1YKV54W

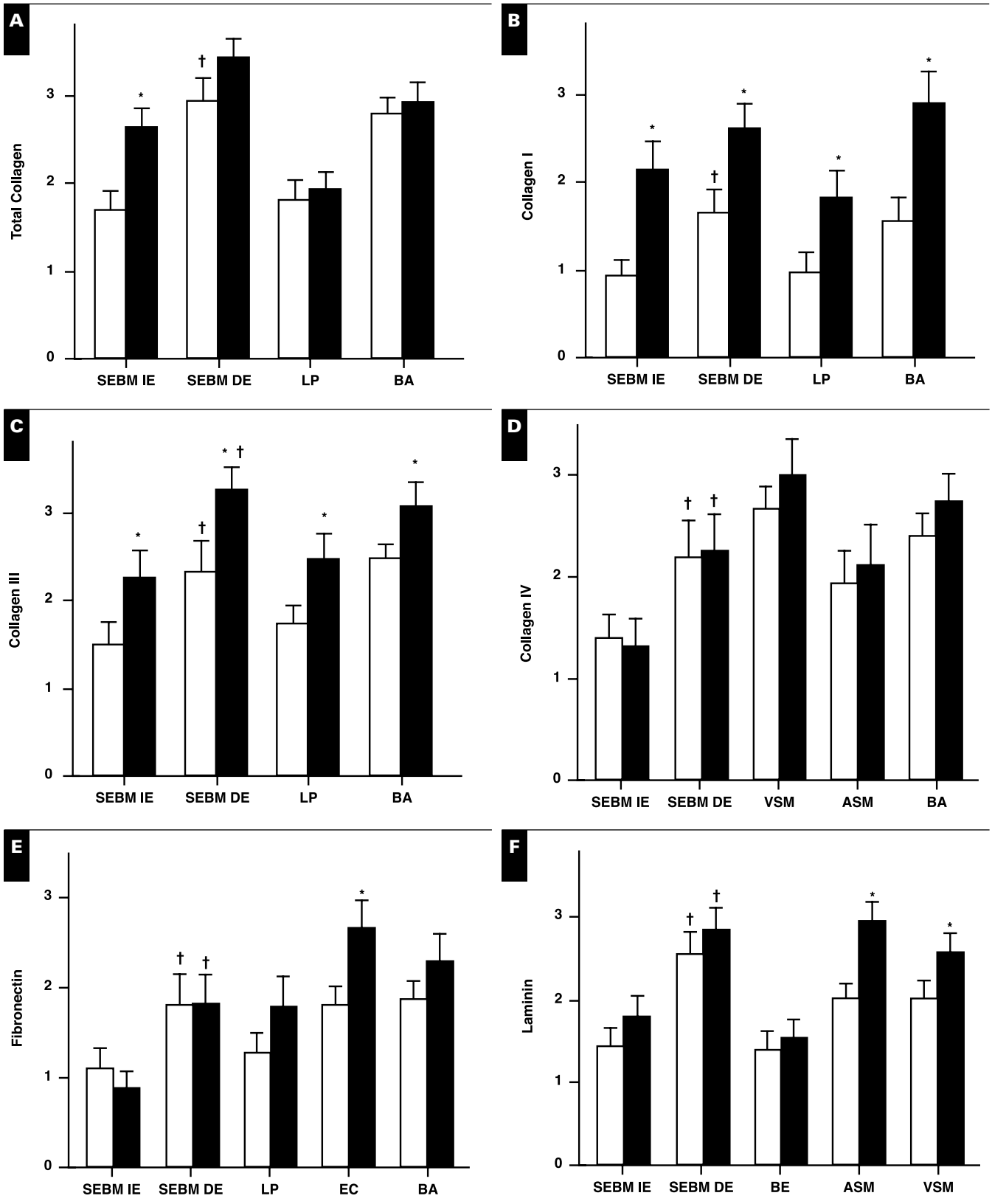


Figure 1 Graphic representations of extracellular matrix proteins (mean ± SEM) using visual scoring for total collagen (**A**), for collagen I (**B**), for collagen III (**C**), for collagen IV (**D**), for fibronectin (**E**), and for laminin (**F**). Staining score for non–chronic obstructive pulmonary disease (COPD) (white bars) and COPD groups (black bars) are given. * $P < .05$ vs the non-COPD group. † $P < .05$ SEBM scores of damaged vs undamaged bronchial epithelium. ASM, airway smooth muscle cells; BA, bronchial adventitia; BE, bronchial epithelium; DE, damaged bronchial epithelium; EC, endothelial cells; IE, intact bronchial epithelium; LP, lamina propria; SEBM, surface epithelial basement membrane; VSM, vascular smooth muscle cells.

DOI: 10.1309/JC477FAEL1YKV54W

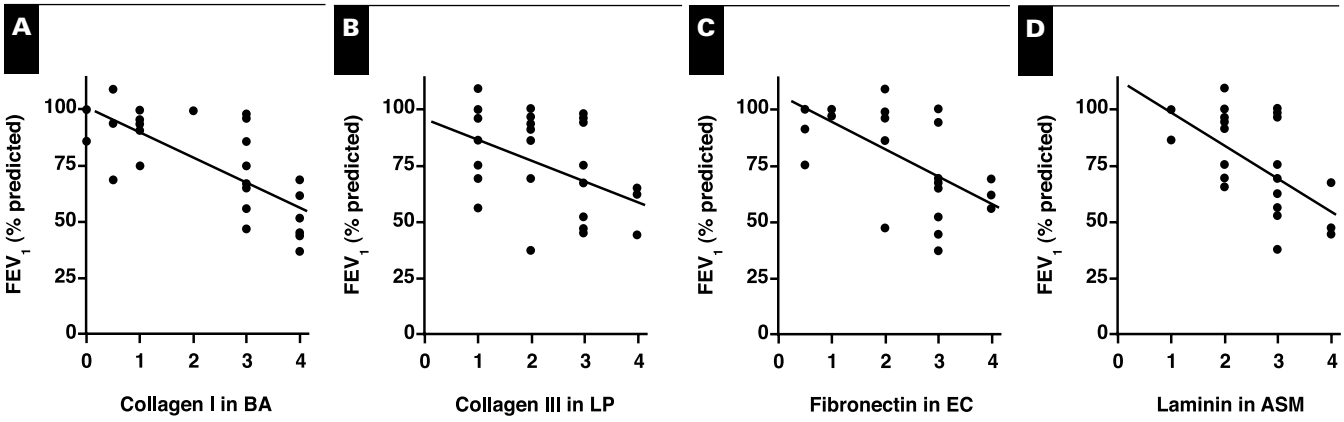


Figure 2 Correlation with FEV_1 (% predicted) of total collagen in surface epithelial basement membrane with undamaged bronchial epithelium (**A**, $r = -0.67$; $P < .001$), collagen III in the lamina propria (**B**, $r = -0.48$; $P < .01$), fibronectin in EC (**C**, $r = -0.51$; $P < .01$) and laminin in VSM (**D**, $r = -0.61$; $P < .001$) of the combined patient groups (non–chronic obstructive pulmonary disease [COPD] and COPD). The correlation coefficient (r) was obtained using linear regression (Pearson) analysis and significance level ($P < .05$) was considered at absolute values of $r > 0.37$. Staining scores of 0, 1, 2, 3, and 4 represent no, weak, moderate, intense, and very intense staining, respectively. ASM, airway smooth muscle cells; BA, bronchial adventitia; EC, endothelial cells; FEV_1, forced expiratory volume in 1 second; LP, lamina propria.

of elastic recoil in the peripheral airways and lung parenchyma of patients with COPD.[6,23] Inflammation, with influx of CD8+ T cells in peripheral airways and accumulation of macrophages has been reported.[5,24] Peribronchiolar and septal fibrosis also are found, whereas alveolar ECM deposition is decreased in emphysema.[5,24]

Our results demonstrate that COPD also is associated with changes in ECM protein deposition of larger airways. In asthma, SEBM thickening is prominent, as is the deposition in large airways of various ECM proteins, including collagens, fibronectin, laminins, and proteoglycans in epithelial SEBM, subepithelial layers, and bronchial vasculature.[25-27] In COPD, however, the few reports that are available have indicated that SEBM thickness remains unchanged, unless features of asthma such as hyperresponsiveness or corticosteroid sensitivity were present.[3,13] However, we show here that the staining of total collagen, collagen I, and collagen III in SEBM is more intense in COPD than in control subjects. Furthermore, all studied ECM proteins were up-regulated at sites where the epithelial lining was damaged. These findings support the hypothesis of involvement of the bronchial epithelium and subepithelial myofibroblasts in damage and repair processes with tissue remodeling.

Studies based on in vitro coculture experiments indicate that effects of growth factors such as epidermal growth factor, fibroblast growth factors 1 and 2, and transforming growth factor β1 on epithelial cells and myofibroblasts are necessary to mediate repair of epithelial injury by induction of cellular proliferation and collagen synthesis.[28-31] The aforementioned mechanisms that were found in vitro possibly could also have a role in tissue remodeling and fibrosis during COPD. We also studied the deposition of ECM proteins in the bronchial vasculature of the bronchial lamina propria and adventitia. We found that COPD is associated with more deposition of collagen III and laminin in vascular media and adventitia and with fibronectin in endothelial cells and also the neointima of small muscular vessels. We and others have previously shown that structural changes to the pulmonary vasculature, including intimal and medial thickening with vascular smooth muscle (VSM) hypertrophy and lumen narrowing, occur in COPD.[16,19,32] We described that in the peripheral lung, vessel wall thickness was correlated inversely with FEV_1. Peinado et al[33,34] concluded that small pulmonary arteries of patients with mild COPD have endothelial dysfunction and intimal thickening.

In the present study, we used the method of visual scoring on immunohistochemically stained bronchial tissue sections, which also was used by Santos et al[19] and others[10,35] for the staining pattern of various ECM components, including elastin, total collagen, and proteoglycans. Santos and colleagues[19] reported no differences in small pulmonary arteries between COPD patients and smoking non-COPD control subjects, but a positive correlation between the amount of collagen deposition and intimal thickening was found. Analogous to the ECM deposition in the bronchial wall, damage to the endothelial lining can induce vascular remodeling, VSM proliferation, metaplasia of VSM to myofibroblasts, and increased synthesis and deposition of ECM proteins such as collagens and fibronectin.[36] Our results support this hypothesis, which likely also contributes to vascular remodeling during the development of COPD.

DOI: 10.1309/JC477FAEL1YKV54W

Correlation analysis revealed a significant inverse correlation of FEV_1 values and total collagen and collagen I and III staining in the SEBM, fibronectin in the intima of mucosal vessels, and laminin expression in ASM. Focus is now on the bidirectional relationship between ASM cells and the ECM. In addition to increased synthesis of ECM proteins, ASM cells can be involved in the down-regulation of matrix metalloproteinases and the up-regulation of tissue inhibitors of metalloproteinases.[37] These findings are consistent with the hypothesis of the development of structural abnormalities in the bronchial airway wall and in the vessel walls in patients with COPD causing airway obstruction. Taken together, our results indicate that COPD is associated with increased deposition of ECM components in the bronchial airway wall as part of airway remodeling and contributing to airflow limitation. Blockade of pathways that are likely to be involved in structural and functional abnormalities should be considered in the development of therapeutic interventions aimed to prevent chronic airflow limitation in COPD.

From the Departments of [1]Pharmacology, Erasmus MC, University Medical Centre, Rotterdam; [2]Pathology, VUMC, University Medical Centre, Amsterdam; and [3]Pulmonology, Leiden University Medical Centre, Leiden, the Netherlands.

Supported by research grant NAF 32.97.73 from the Netherlands Asthma Foundation, Leusden.

Address reprint requests to Dr Sharma: Cardiopulmonary and Molecular Biology Lab, Dept of Pharmacology, Erasmus MC, University Medical Center, Dr Molewaterplein 50, 3015 GE Rotterdam, the Netherlands.

Acknowledgment: We thank P.R. Saxena, MD, for critically reading the manuscript.

References

1. Celli BR, MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J*. 2004;23:932-946.
2. Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med*. 2001;164(10 pt 2):S28-S38.
3. Rutgers SR, Timens W, Kauffman HF, et al. Markers of active airway inflammation and remodelling in chronic obstructive pulmonary disease. *Clin Exp Allergy*. 2001;31:193-205.
4. Kuwano K, Bosken CH, Pare PD, et al. Small airways dimensions in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis*. 1993;148:1220-1225.
5. Saetta M, Di Stefano A, Turato G, et al. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1998;157(3 pt 1):822-826.
6. Hogg JC, Chu F, Utokaparch S, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med*. 2004;350:2645-2653.
7. Tiddens HA, Pare PD, Hogg JC, et al. Cartilaginous airway dimensions and airflow obstruction in human lungs. *Am J Respir Crit Care Med*. 1995;152:260-266.
8. Di Stefano A, Capelli A, Lusuardi M, et al. Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med*. 1998;158:1277-1285.
9. Vignola AM, Chanez P, Chiappara G, et al. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med*. 1997;156(2 pt 1):591-599.
10. Kranenburg AR, Willems-Widyastuti A, Mooi WJ, et al. Chronic obstructive pulmonary disease is associated with enhanced bronchial expression of FGF-1, FGF-2, and FGFR-1. *J Pathol*. 2005;206:28-38.
11. Amin K, Ludviksdottir D, Janson C, et al. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. *Am J Respir Crit Care Med*. 2000;162:2295-2301.
12. Fabbri LM, Romagnoli M, Corbetta L, et al. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2003;167:418-424.
13. Chanez P, Vignola AM, O'Shaugnessy T, et al. Corticosteroid reversibility in COPD is related to features of asthma. *Am J Respir Crit Care Med*. 1997;155:1529-1534.
14. Grashoff WF, Sont JK, Sterk PJ, et al. Chronic obstructive pulmonary disease: role of bronchiolar mast cells and macrophages. *Am J Pathol*. 1997;151:1785-1790.
15. de Boer WI, van Schadewijk A, Sont JK, et al. Transforming growth factor beta1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1998;158:1951-1957.
16. Kranenburg AR, De Boer WI, Van Krieken JH, et al. Enhanced expression of fibroblast growth factors and receptor FGFR-1 during vascular remodeling in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol*. 2002;27:517-525.
17. Quanjer PH, Tammeling GJ, Cotes JE, et al. Lung volumes and forced ventilatory flows: report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal: official statement of the European Respiratory Society. *Eur Respir J Suppl*. 1993;16:5-40.
18. Peters TH, de Jong PL, Klompe L, et al. Right ventricular collagen and fibronectin levels in patients with pulmonary atresia and ventricular septal defect. *Mol Cell Biochem*. 2003;251:27-32.
19. Santos S, Peinado VI, Ramirez J, et al. Characterization of pulmonary vascular remodelling in smokers and patients with mild COPD. *Eur Respir J*. 2002;19:632-638.
20. Durieu I, Peyrol S, Gindre D, et al. Subepithelial fibrosis and degradation of the bronchial extracellular matrix in cystic fibrosis. *Am J Respir Crit Care Med*. 1998;158:580-588.
21. Roche WR, Beasley R, Williams JH, et al. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet*. 1989;1:520-524.
22. Sinkin RA, Roberts M, LoMonaco MB, et al. Fibronectin expression in bronchopulmonary dysplasia. *Pediatr Dev Pathol*. 1998;1:494-502.
23. Wright JL, Lawson LM, Pare PD, et al. Morphology of peripheral airways in current smokers and ex-smokers. *Am Rev Respir Dis*. 1983;127:474-477.
24. Moon J, du Bois RM, Colby TV, et al. Clinical significance of respiratory bronchiolitis on open lung biopsy and its relationship to smoking related interstitial lung disease. *Thorax*. 1999;54:1009-1014.
25. Brewster CE, Howarth PH, Djukanovic R, et al. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol*. 1990;3:507-511.

DOI: 10.1309/JC477FAEL1YKV54W

26. Altraja A, Laitinen A, Virtanen I, et al. Expression of laminins in the airways in various types of asthmatic patients: a morphometric study. *Am J Respir Cell Mol Biol.* 1996;15:482-488.
27. Christie PE, Jonas M, Tsai CH, et al. Increase in laminin expression in allergic airway remodelling and decrease by dexamethasone. *Eur Respir J.* 2004;24:107-115.
28. Dube J, Chakir J, Laviolette M, et al. In vitro procollagen synthesis and proliferative phenotype of bronchial fibroblasts from normal and asthmatic subjects. *Lab Invest.* 1998;78:297-307.
29. Holgate ST, Davies DE, Lackie PM, et al. Epithelial-mesenchymal interactions in the pathogenesis of asthma. *J Allergy Clin Immunol.* 2000;105(2 pt 1):193-204.
30. Puddicombe SM, Polosa R, Richter A, et al. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. *FASEB J.* 2000;14:1362-1374.
31. Morishima Y, Nomura A, Uchida Y, et al. Triggering the induction of myofibroblast and fibrogenesis by airway epithelial shedding. *Am J Respir Cell Mol Biol.* 2001;24:1-11.
32. Magee F, Wright JL, Wiggs BR, et al. Pulmonary vascular structure and function in chronic obstructive pulmonary disease. *Thorax.* 1988;43:183-189.
33. Peinado VI, Barbera JA, Ramirez J, et al. Endothelial dysfunction in pulmonary arteries of patients with mild COPD. *Am J Physiol.* 1998;274(6 pt 1):L908-L913.
34. Peinado VI, Barbera JA, Abate P, et al. Inflammatory reaction in pulmonary muscular arteries of patients with mild chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 1999;159(5 pt 1):1605-1611.
35. Shehata SM, Tibboel D, Sharma HS, et al. Impaired structural remodelling of pulmonary arteries in newborns with congenital diaphragmatic hernia: a histological study of 29 cases. *J Pathol.* 1999;189:112-118.
36. Voelkel NF, Cool C, Taraceviene-Stewart L, et al. Janus face of vascular endothelial growth factor: the obligatory survival factor for lung vascular endothelium controls precapillary artery remodeling in severe pulmonary hypertension. *Crit Care Med.* 2002;30(5 suppl):S251-S256.
37. Parameswaran K, Willems-Widyastuti A, Alagappan VK, et al. Role of extracellular matrix and its regulators in human airway smooth muscle biology. *Cell Biochem Biophys.* 2006;44:139-146.

DOI: 10.1309/JC477FAEL1YKV54W