
Effect of extracellular matrix composition on airway epithelial cell and fibroblast structure: implications for airway remodeling in asthma

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Background: Airway remodeling in asthma is characterized by structural changes to the airways including extracellular matrix (ECM) deposition and epithelial metaplasia. Extracellular matrix deposition in the subepithelial region may play an important role in modulation of epithelial cell and fibroblast structure and function because it lies in immediate contact with these cell types and exists within the functional epithelial mesenchymal trophic unit.

Objective: To investigate the effect of aberrant ECM components on airway epithelial cells and fibroblasts and the relationship among subepithelial ECM deposition, other remodeling changes, and airway hyperresponsiveness.

Methods: BEAS-2B human airway epithelial cells and WI-38 human airway fibroblast cells were cultured on various ECM protein substrates (Matrigel, representing normal basement membrane matrix, or aberrant matrix proteins collagen I, collagen III, and fibronectin). Airway remodeling changes were determined using morphometry in sections from a murine model of chronic allergic airway disease. Airway reactivity to methacholine was determined, and these parameters correlated.

Results: Abnormal ECM substrates induced epithelial and fibroblast proliferation and altered the cell morphology of both human airway epithelial cells and fibroblasts when compared with normal basement membrane ECM. Subepithelial matrix deposition in the mouse correlated with epithelial thickness, but only weak correlations were noted among the other parameters.

Conclusions: We have demonstrated that ECM may affect the growth of airway epithelial cells and fibroblasts in vitro and may influence epithelial thickness in the mouse. These findings may have implications for understanding the pathogenesis of asthma and future therapeutic targeting of airway remodeling.

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INTRODUCTION

Structural alterations to the airway wall in asthma are known as airway remodeling. They include changes such as goblet cell hyperplasia, epithelial shedding, deposition of extracellular matrix (ECM) proteins beneath the basement membrane (BM) in the reticular BM (RBM), myofibroblast differentiation, smooth muscle thickening, and angiogenesis.¹ Airway remodeling is important because there is strong evidence to suggest that it is associated with severe and steroid-resistant disease subsets and contributes to fixed airway obstruction, irreversible loss of lung function, and increased airway hyperresponsiveness (AHR).^{1–5}

The origin of airway remodeling in asthma remains poorly understood, and it may occur independently of airway inflammation.⁶ One theory gaining increasing acceptance is that damage to the epithelium results in a defective repair phenotype with the release of the proinflammatory mediators that can initiate and perpetuate the airway remodeling process.^{7,8}

Regardless of cause, there is clear evidence that both repeated episodes of airway inflammation and the interaction between individual airway remodeling components can drive the remodeling process. There is also evidence of remodeling demonstrated in infants, children, and patients with rhinitis with atopy before the development of symptomatic asthma.⁹ We have shown in animal models of allergic airway disease (AAD) that airway fibrosis occurs early in the sequence of structural changes to the airway.³ This suggests that airway fibrosis may play a role in progression of other remodeling changes involving the epithelium and fibroblasts.

Airway fibrosis or increased ECM deposition has been identified as an important factor contributing to the pathogenesis of severe asthma. The RBM is significantly thickened even in mild forms of the disease¹⁰ and is phenotypically different from the normal BM. Reticular BM thickening is related to other airway remodeling changes and correlates with airflow limitation and AHR and is negatively correlated with airway distensibility.^{10–13} Although the normal airway BM is composed primarily of laminin, entactin, and collagen IV,^{14,15} the abnormal RBM consists predominantly of collagen I, collagen III, fibronectin, tenascin, laminin, and proteoglycans.^{16,17}

Transforming growth factor β (TGF- β) plays a key role in the regulation of fibrosis. It has been shown to activate and promote myofibroblast survival,^{18,19} collagen synthesis,²⁰ and

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fibronectin and tenascin synthesis.^{21,22} Furthermore, TGF- β inhibits ECM degradation by inhibiting matrix metalloproteinase synthesis²³ and increasing the synthesis of tissue inhibitors of matrix metalloproteinases.

Coculture experiments have demonstrated interactions between airway epithelial and fibroblast cells. Damage to bronchial epithelial cells (BECs) led to fibroblast proliferation and increased secretion of profibrotic growth factors, basic fibroblast growth factor, platelet-derived growth factor, TGF- β 2, and endothelin 1.²⁴ Myerburg and colleagues grew primary BECs with airway fibroblasts in a medium that promoted squamous differentiation and found that the presence of fibroblasts induced polarized ciliated differentiation and accelerated wound repair.²⁵ Studies have also suggested effects of specific ECM proteins on epithelial and fibroblast cells. Studies using rat tracheal epithelial cells showed the collagen I-affected epithelial differentiation.²⁶ In a separate study, the cell density of lung epithelial cells cultured from rat fetuses was higher when grown on collagen I and fibronectin when compared with collagen IV.²⁷

The objective of the current study was to investigate how aberrant ECM components may affect the growth and phenotype of airway epithelial cells and fibroblasts. Cells were seeded onto Matrigel as a normal BM control or onto abnormal ECM substrates (collagen I, collagen III, or fibronectin), and cell proliferation and cell differentiation were evaluated. The correlation between ECM deposition and epithelial remodeling and AHR was also investigated using archived lung tissue from mice with chronic AAD.

METHODS

Cell Lines

Two cell lines were used in the in vitro cell culture system: human BEC line BEAS-2B (American Type Culture Collection No. CRL-9609) and fibroblast cell line WI-38 (American Type Culture Collection No. CCL-75). The BEAS-2B sample was derived from epithelial cells isolated from normal human bronchial epithelium obtained from noncancerous individuals, and the WI-38 sample was obtained from human embryonic lung tissue.^{28,29}

The BEAS-2B cells were cultured in a 1:1 preparation of minimum essential medium supplemented with 10% fetal calf serum and 1% penicillin-streptomycin and keratinocyte serum-free medium (Invitrogen, Melbourne, Australia) and maintained in a humidified atmosphere of 5% carbon dioxide (CO₂) in air, at 37°C. The WI-38 fibroblasts were grown up to confluence in minimum essential medium supplemented as described, but without keratinocyte serum-free medium, and maintained in a humidified atmosphere of 5% CO₂ in air, at 37°C.

ECM Substrates

Normal BM matrix (Matrigel; BD Biosciences, San Jose, California) was used as a model of normal BM.¹⁷ It contains laminin, collagen IV, entactin, and some growth factors, in similar amounts to that present in a normal BM.^{30,31}

To mimic an environment in which subepithelial fibrosis occurs, 6-well plates (Corning Life Sciences, New South Wales, Australia) were coated with 1 of 3 ECM substrates: collagen I from human placenta, collagen III from human placenta, or human recombinant fibronectin (Chemicon, Temecular, California; and Invitrogen). Plates were allowed to air dry for 3 hours.

Six-well plates were coated with normal BM matrix at a concentration of 450 μ L per well (50 μ L/cm²), in accordance with the manufacturer's instructions, and subsequently incubated for at least 2 hours at 37°C.

After coating of plates, either WI-38 or BEAS-2B cells were seeded at a density of 5×10^5 cells per well onto normal BM matrix or aberrant ECM in triplicate, in 2 mL of growth media per well, and cultured for 6 days at 37°C, 5% CO₂, in air. Three separate experiments were performed.

Cell Proliferation

Cell proliferation was evaluated by determining total cell count on day 6. Total viable cell counts were performed in triplicate for each of the 2 cell lines in each of the culture conditions previously outlined, using a hemocytometer and Trypan blue exclusion. A minimum of 500 cells were counted. Results were validated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay.³²

Cell Morphology

Cell morphology for each of the cell lines on each of the different surfaces was evaluated by phase contrast microscopy, on days 2 and 6. Changes in cell morphology were noted on each day, and images were captured with a digital camera. The extent of branching (cell-to-cell processes) was evaluated by calculating the mean number of cell branches per fibroblast on each substrate.

Cell Phenotype of Epithelial Cells

To determine if ECM substrate induced a goblet cell phenotype, immunohistochemical and histochemical staining was performed for known markers of airway goblet cells. The BEAS-2B epithelial cells grown on the substrates described were pelleted, fixed overnight in formalin, and embedded in paraffin. Sections were stained immunohistochemically using a 4-stage procedure, as previously described.^{33,34} The monoclonal antibody to MUC5AC (clone 45M1; Novacastra Laboratories, Newcastle upon Tyne, England) was used at a dilution of 1:50, and polyclonal antibody to trefoil factor 2 (TFF2) was used at a dilution of 1:1,000.³⁵ Alcian blue-periodic acid-Schiff (AB-PAS) staining was used to demonstrate neutral and acidic mucin staining.

TGF- β Enzyme-Linked Immunosorbent Assay

To quantitate levels of TGF- β secreted from either epithelial cells or fibroblasts, TGF- β levels in cell culture supernatants on days 2 and 6 were determined by an enzyme-linked immunosorbent assay (ELISA), human TGF- β 1 ELISA kit (BD Biosciences, Franklin Lakes, NJ), according to the manufacturer's instructions.

Correlation Analysis of the Relationship Between ECM Deposition and Airway Remodeling and AHR in a Mouse Model of AAD

To analyze the relationship between deposition of ECM and epithelial thickening and goblet cell hyperplasia, morphometric analysis was conducted on Masson trichrome and AB-PAS-stained sections from mice, collected as part of a previous study of chronic AAD.³ Mean thicknesses were determined as previously described using Image-Pro Discovery Software (Media Cybernetics, Silver Spring, Maryland).³

Photomicrographic images were captured using a Spot Cooled Color Digital camera (Q Imaging, Burnaby, British Columbia). Briefly, a minimum of 5 bronchi, measuring 150 to 350 μm in luminal diameter, were analyzed per mouse for the parameters described subsequently herein. The thickness of the bronchial epithelial layer was measured in hematoxylin-eosin-stained sections. Subepithelial deposition of collagen was measured in sections stained with Masson's trichrome. Hyperplasia of goblet cells within the bronchial epithelium was evaluated by counting cells in AB-PAS-stained sections and expressed as the mean number of goblet cells per 100 μm of BM.

Methacholine-induced airway reactivity was evaluated by direct plethysmography (Buxco Electronics, Troy, New York). Increasing methacholine doses were delivered intravenously, and airway resistance and dynamic compliance were measured (Biosystem XA; Buxco Electronics) for 2 minutes after each dose. Results were expressed as the maximal resistance after each dose of methacholine minus baseline (phosphate-buffered saline alone) resistance.³

Statistical Analysis

Results from the proliferation studies (cell counts and mean number of branches per fibroblast) were analyzed by *t* test. $P < .05$ was considered statistically significant. Data analysis was performed using a software package (GraphPad Prism 4.0; GraphPad Software, San Jose). Pearson correlation coefficients (*r*) were used to estimate correlations.

RESULTS

Proliferation of BECs

Proliferation was evaluated on day 6. When seeded on normal BM matrix, epithelial cells proliferated at an average rate of 7.58×10^4 cells per day within the first 2 days of the experiment. By days 5 and 6, the cells were proliferating at approximately 10.00×10^4 cells per day. The MTT assay results showed proliferation consistent with these cell counts. A significant increase in proliferation of BEAS-2B cells was observed on collagen I (mean, 5.1×10^5 cells; SD, 2.7×10^5 cells; $P < .001$), collagen III (mean, 4.5×10^5 cells; SD, 2.1×10^5 cells; $P < .001$), and fibronectin (mean, 4.6×10^5 cells; $P < .001$) substrates compared with the normal BM control (mean, 2.1×10^5 cells; SD, 6.0×10^4 cells) (Fig 1A).

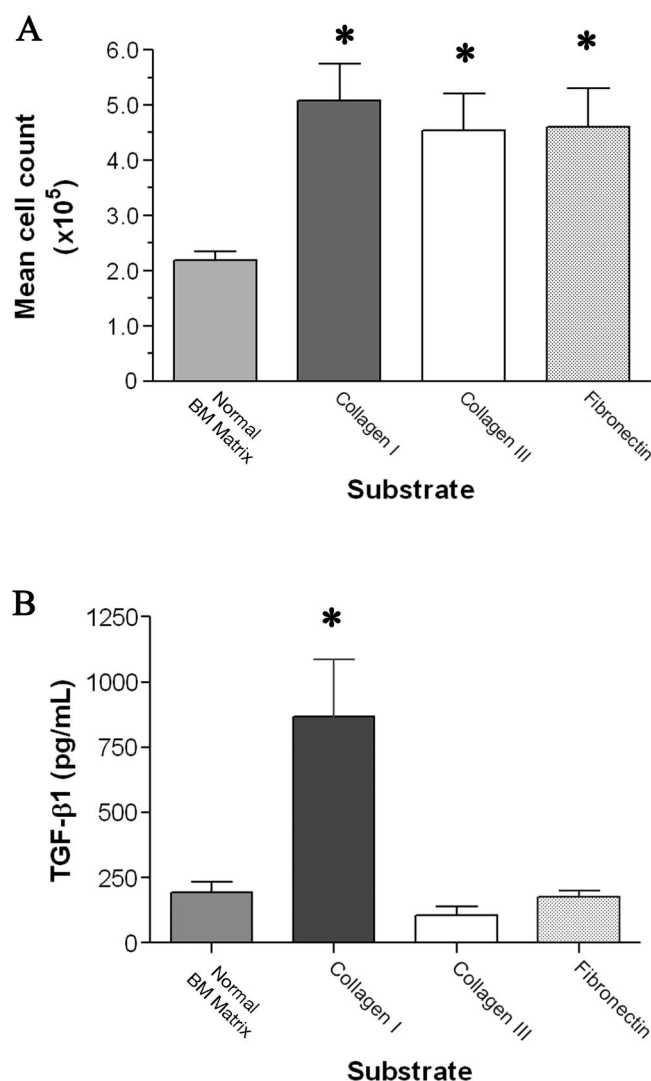


Figure 1. A, Proliferation of BEAS-2B cells on normal basement membrane (BM) matrix (Matrigel), collagen I, collagen III, and fibronectin on day 6. The graph represents mean (SEM) data from 3 separate experiments. Cell growth was accelerated on collagens I and III and fibronectin, compared with the BM control, suggesting that reticular BM composition affects epithelial proliferation in the airway. * $P < .001$ vs BM control. B, Levels of transforming growth factor (TGF) $\beta 1$ secreted into cell culture supernatant by BEAS-2B cells after 6 days of culture on normal BM matrix, collagen I, collagen III, and fibronectin substrates, quantitated by enzyme-linked immunosorbent assay. The graph represents mean (SEM) data from 3 separate experiments. Secretion of TGF- $\beta 1$ increased dramatically when epithelial cells were seeded on a collagen I substrate. However, cells on collagen III and fibronectin substrates showed no significant difference in levels of TGF- $\beta 1$. * $P < .001$ vs BM control.

Morphology of BECs

Changes in morphology were evaluated during the 6 days of culture. During the 6-day culture period on normal BM matrix, epithelial cells became narrower and adopted an elon-

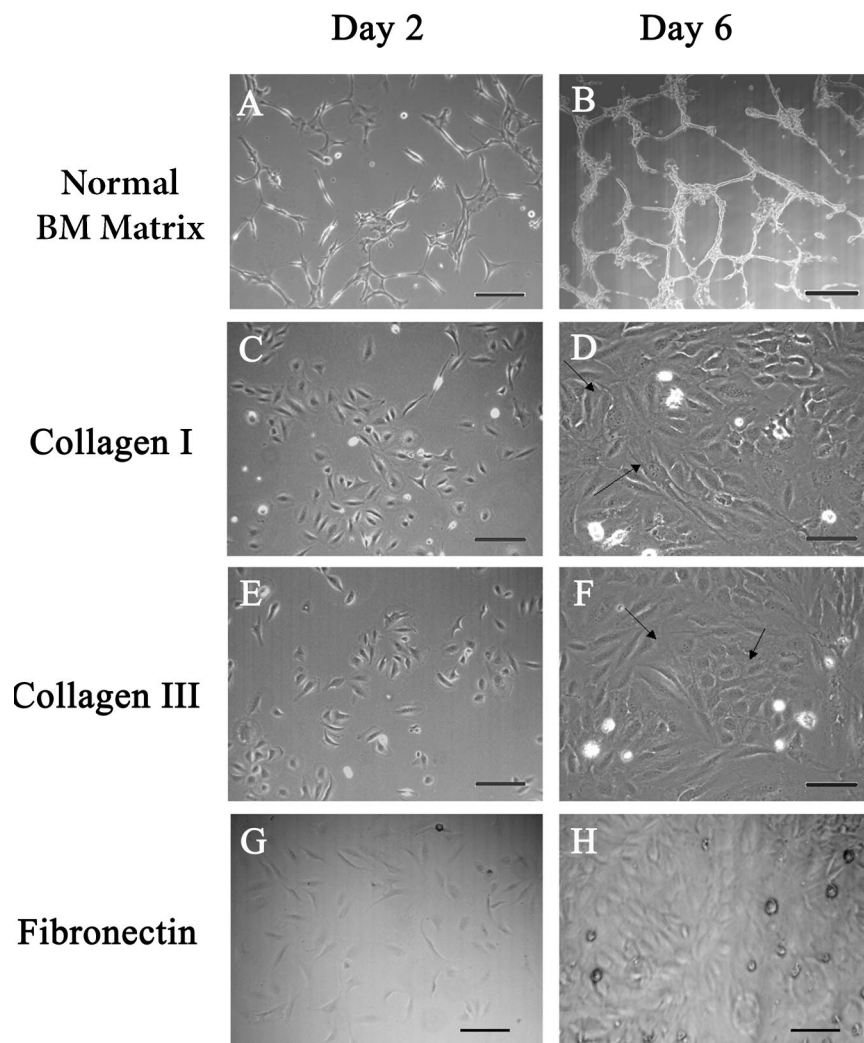


Figure 2. Phase contrast microscopy showing morphology of BEAS-2B cells on days 2 and day 6 on normal basement membrane (BM) matrix (Matrigel)-, collagen I-, collagen III-, and fibronectin-coated surfaces. A, Normal BM matrix, day 2. Epithelial cells begin to adhere in reticular formations. B, Normal BM matrix, day 6. Alveolarlike structures are observed. C, Collagen I, day 2. Epithelial cells adopt a rounder, polarized cell morphology. D, Collagen I, day 6. Granules are present, as indicated by arrows. E, Collagen III, day 2. Cells adopt a similar shape as those on the collagen I substrate, with early clustering. F, Collagen III, day 6. Granules are present (arrows). Two distinct cell shapes (elongated and ovoid and a more rounded shape) are apparent. G, Fibronectin, day 2. An elongated cell shape is observed. H, Fibronectin, day 6. Most of the epithelial cells assume a rounded cell morphology. The bar equals 100 μm .

gated shape similar in appearance to fibroblasts (Fig 2). On day 2, the cells began to arrange themselves in reticular formations; by day 6, the extent of reticular formation had increased by at least 3-fold, with large alveolarlike structures spanning 80 to 120 μm .

Cell differentiation was noted when cells were cultured on ECM proteins, as indicated by a number of changes in cell morphology. First, epithelial cells adopted a rounded cell morphology, when seeded onto surfaces coated with collagen

I and III and fibronectin, compared with the BM control (Fig 2). This was observed as more cells developing a rounder shape and an increased cytoplasm to nucleus ratio. However, the BEAS-2B cells did not stain for acidic or neutral mucins with AB-PAS staining, MUC5AC mucin, or TFF2.

TGF- β 1 Expression in BECs

The TGF- β 1 ELISA revealed low concentrations of TGF- β 1 secreted by epithelial cells into the supernatant, when seeded on normal BM matrix. A mean of 192-pg/mL TGF- β 1 (SD, 69.36 pg/mL) was secreted during the 6-day period of each experiment (Fig 1B).

The BEAS-2B cells grown on a collagen I substrate secreted significantly more TGF- β 1 (mean, 867.1 pg/mL; SD, 379.3 pg/mL) compared with the normal BM control ($P = .04$) and collagen III ($P = .03$).

Proliferation of Lung Fibroblasts

Lung fibroblasts seeded on a normal BM matrix substrate proliferated at an average rate of 2.56×10^4 cells per day within the first 2 days. This was almost 3 times slower than the proliferative rate of epithelial cells grown on normal BM matrix.

Lung fibroblasts proliferated significantly on collagen I (mean, 3.4×10^5 cells; SD, 6.3×10^4 cells; $P < .001$), collagen III (mean, 2.2×10^5 cells; SD, 7.0×10^4 cells; $P < .001$), and fibronectin (mean, 2.3×10^5 cells; SD, 9.5×10^4 cells; $P < .001$) substrates compared with the control BM ECM mixture (mean, 6.9×10^4 cells; SD, 2.3×10^4 cells) (Fig 3A). Furthermore, the proliferation rate on a collagen I substrate was significantly greater than that on collagen III ($P = .002$). The MTT assay results showed proliferation consistent with these cell counts.

Morphology of Lung Fibroblasts

Fibroblasts maintained their typical fusiform morphology when grown on a normal BM, with cellular processes developing on day 2 (Fig 4). From days 3 to 5, these processes (branches) grew and adhered to adjacent cells, creating a network of fibroblasts. On day 6, an extensive syncytium of fibroblasts was formed with cells arranged in parallel. The fibroblasts had a mean of 1.40 branches per cell (SD, 1.083 branches per cell) (Fig 3B). Lung fibroblasts did not exhibit

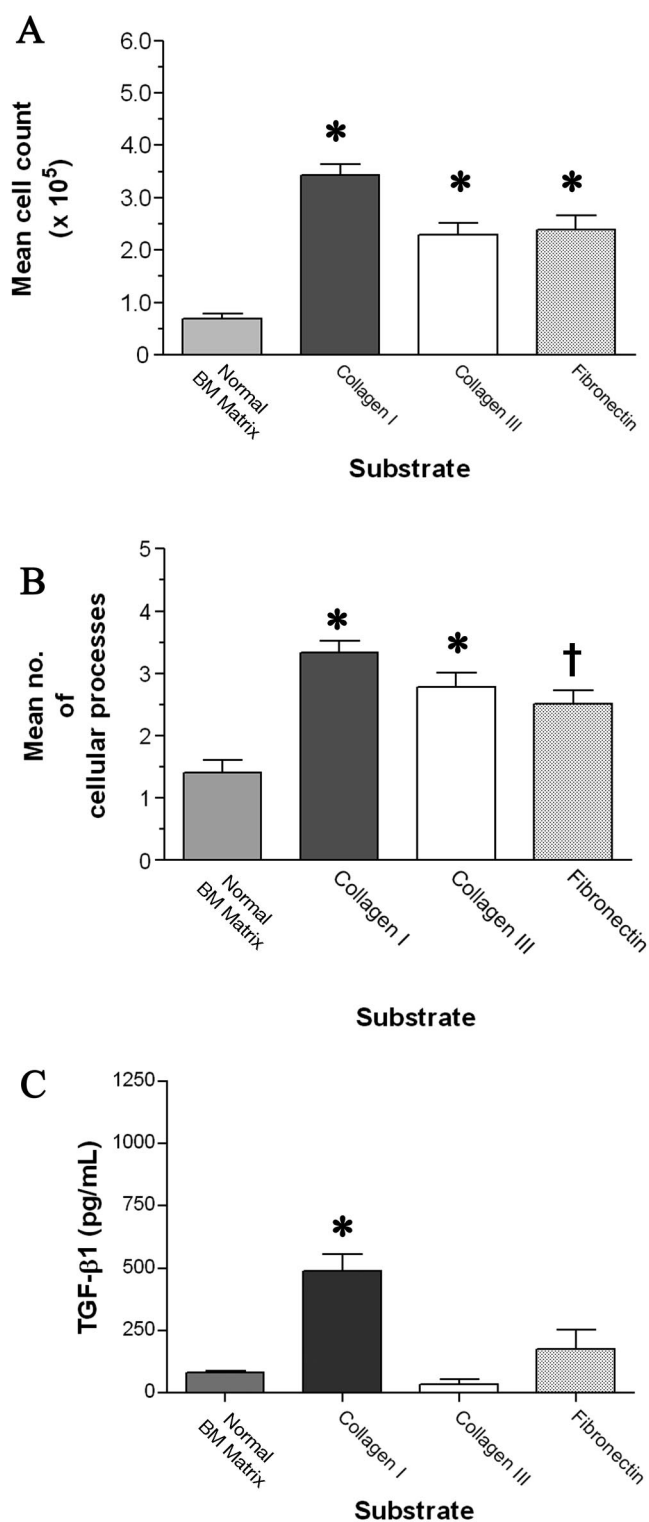


Figure 3. A, Proliferation of WI-38 cells on normal basement membrane (BM) matrix (Matrigel), collagen I, collagen III, and fibronectin on day 6. The graph represents mean (SEM) data from 3 separate experiments. Cell growth was accelerated on collagen I, collagen III, and fibronectin compared

any noticeable changes in morphology when grown on collagen I and III (Fig 4C-F). The arrangement of the fibroblasts was maintained, such that fibroblasts ran in parallel on collagen I- and collagen III-coated surfaces. However, the extent of branching increased compared with fibroblasts on a normal BM control, with an average of 3.33 branches (SD, 0.960 branches) per cell on day 6 observed on collagen I ($P < .001$) and 2.78 branches (SD, 1.18 branches) per cell on collagen III ($P < .001$) substrates (Fig 3B).

The arrangement of the fibroblasts altered when seeded on a fibronectin-coated surface with the cells forming glandularlike structures (Fig 4G-H). In addition, the cells also displayed extensive branching, with a mean of 2.52 (SD, 1.05) cellular processes per cell compared with the normal BM control ($P < .001$) (Fig 3B).

TGF-β1 Expression in Lung Fibroblasts

The TGF-β1 was undetectable (lower limit of detection for the assay, <80 pg/mL) in the supernatant of lung fibroblasts grown on a normal BM substrate for 6 days (Fig 3C), which was substantially less than levels secreted by the BECs.

Levels of TGF-β1 were detected in the supernates of fibroblast samples from each substrate. Collagen I substrate augmented the secretory level almost 5-fold (mean, 488.3 pg/mL; SD, 995.46 pg/mL; $P = .03$) compared with the BM control (Fig 3C).

Analysis of Correlation Between ECM Deposition and Epithelial Changes in a Mouse Model of AAD

To investigate the correlation between subepithelial ECM deposition and epithelial remodeling in a mouse model of chronic AAD, the relationship between these parameters was examined by morphometric analysis in Masson trichrome and AB-PAS-stained sections. There was a moderately strong correlation observed between subepithelial ECM thickness and epithelial thickness ($r = 0.68$, $P < .001$, $n = 143$) (Fig 5A). There was a moderate correlation between the number of goblet cells and the mean subepithelial ECM thickness ($r = 0.51$, $P < .001$, $n = 80$) (Fig 5B).

with the BM control, suggesting that reticular BM composition affects fibroblast proliferation in the airway. * $P < .001$ vs BM control. B, Mean number of cellular branches per fibroblast after 6-day culture on BM control, collagen I, collagen III, and fibronectin substrates. The graph represents mean (SD) data from 3 separate experiments on each substrate. The extent of branching increases significantly on abnormal extracellular matrix substrates compared with the BM control. * $P \leq .001$ and † $P = .001$ vs BM control. C, Levels of transforming growth factor (TGF) β1 secreted into cell culture supernatant by WI-38 cells after 6-day culture on BM control, collagen I, collagen III, and fibronectin, as quantitated by enzyme-linked immunosorbent assay. The graph represents mean (SEM) data from 3 separate experiments. A significant increase in the secretion of TGF-β1 was observed on collagen I substrate, but not on collagen III or fibronectin substrates, vs BM control. * $P < .05$ vs BM control.

Analysis of Correlation Between Airway Remodeling Changes and AHR in a Mouse Model of AAD

To investigate the correlation between epithelial and subepithelial airway remodeling changes and AHR in a mouse model of chronic AAD, the relationship between these parameters was examined by morphometric analysis in Masson trichrome-stained sections and methacholine-induced AHR by invasive plethysmography. There was little to no correlation observed between subepithelial ECM thickness and AHR ($r = 0.04$, $P = .74$, $n = 64$) (Fig 6A). There was a weak correlation between epithelial thickness and AHR ($r = 0.25$, $P < .01$, $n = 64$) (Fig 6B).

DISCUSSION

In the field of airway remodeling, there has been much interest in the epithelial mesenchymal trophic unit. This concept emphasizes the interrelationship between epithelial cells and fibroblasts. Evidence has been presented that these cell types interact by release of growth factors and profibrotic signals that influence the epithelial and fibroblast cell compartments and drive other remodeling changes.^{36,37} During airway remodeling, the normal BM is replaced by a thickened RBM comprising abnormal ECM proteins collagen I and III and fibronectin.¹⁶ The results of the current study demonstrate that abnormal ECM components, characteristic of the RBM, may also contribute to driving the remodeling process by modulating proliferation, differentiation, and function of BECs and fibroblasts *in vitro*.

Alongside repeated episodes of allergic inflammation, epithelial damage is regarded as a major etiology of airway remodeling. Goblet cell hyperplasia is mediated via epidermal growth factor R and possibly hepatocyte growth factor R and is characterized by up-regulation of MUC5AC and TFF2.^{38,39} In the current study, a rounded cell morphology was elicited by an aberrant ECM. However, this change was not accompanied by TFF2, MUC5AC, or mucin production.

Researchers have previously shown that fibrosis can drive other airway remodeling changes.¹ Extracellular matrix deposition may affect changes to the epithelium, smooth muscle,

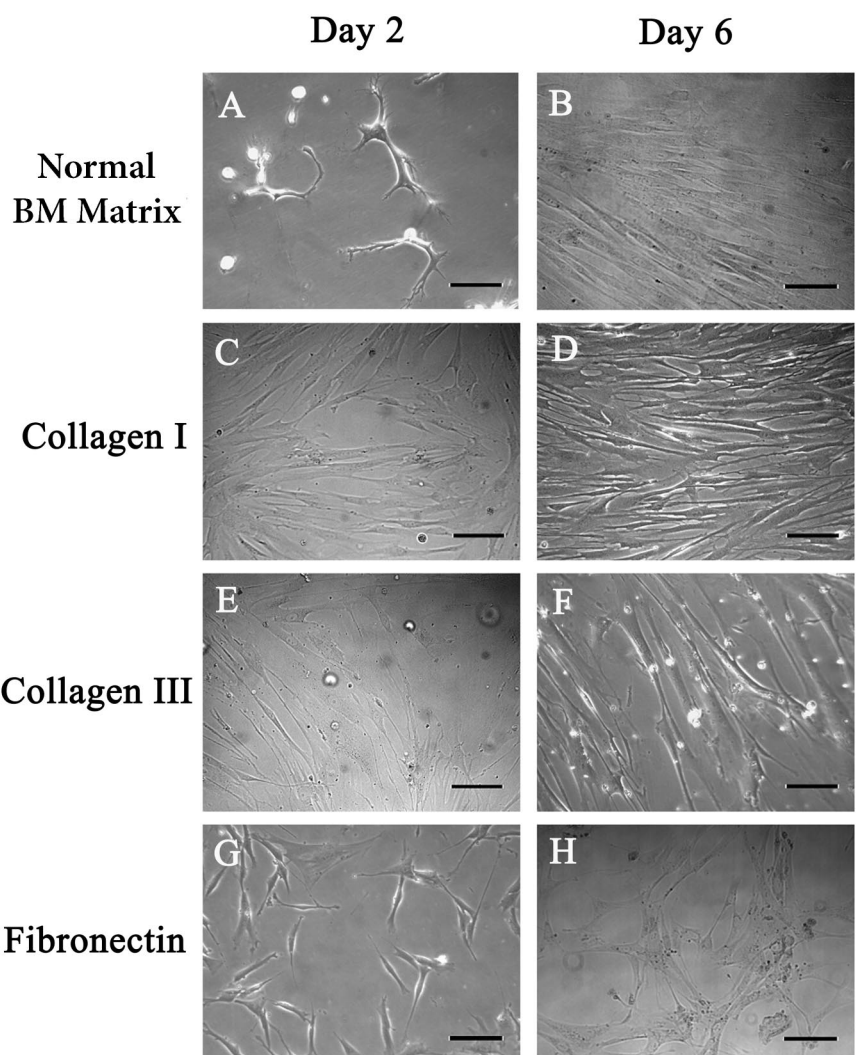


Figure 4. Phase contrast microscopy of lung fibroblasts (WI-38 cells) on days 2 and day 6 grown on normal basement membrane (BM) matrix (Matrigel)-, collagen I-, collagen III-, and fibronectin-coated surfaces. A, Normal BM matrix, day 2. Lung fibroblasts show budding of branches. B, Normal BM matrix, day 6. Cells run closely together and in parallel. C, Collagen I, day 2. Fibroblasts begin to develop long dendritelike processes or branches. D, Collagen I, day 6. Extent of branching increases by day 6. E, Collagen III, day 2. Similar cell morphology and arrangement to that observed on collagen I substrate. F, Collagen III, day 6. Fibroblasts develop numerous cell branches by day 6. G, Fibronectin, day 2. Fibroblasts on fibronectin substrate adopt reticular formations. H, Fibronectin, day 6. Alveolarlike structures can be observed on day 6. The bar equals 100 μm .

and airway vasculature. Studies have shown that RBM fibrosis may drive the progression of remodeling by contributing to airway smooth muscle hyperplasia^{17,40} and bronchial epithelial thickening.⁴¹ In addition, ECM proteins and myofibroblasts can exacerbate inflammation.^{42,43}

TGF- β 1 is a major profibrotic cytokine in airway remodeling. It has been shown that both BECs and fibroblasts grown on collagen I had increased TGF- β 1 expression in

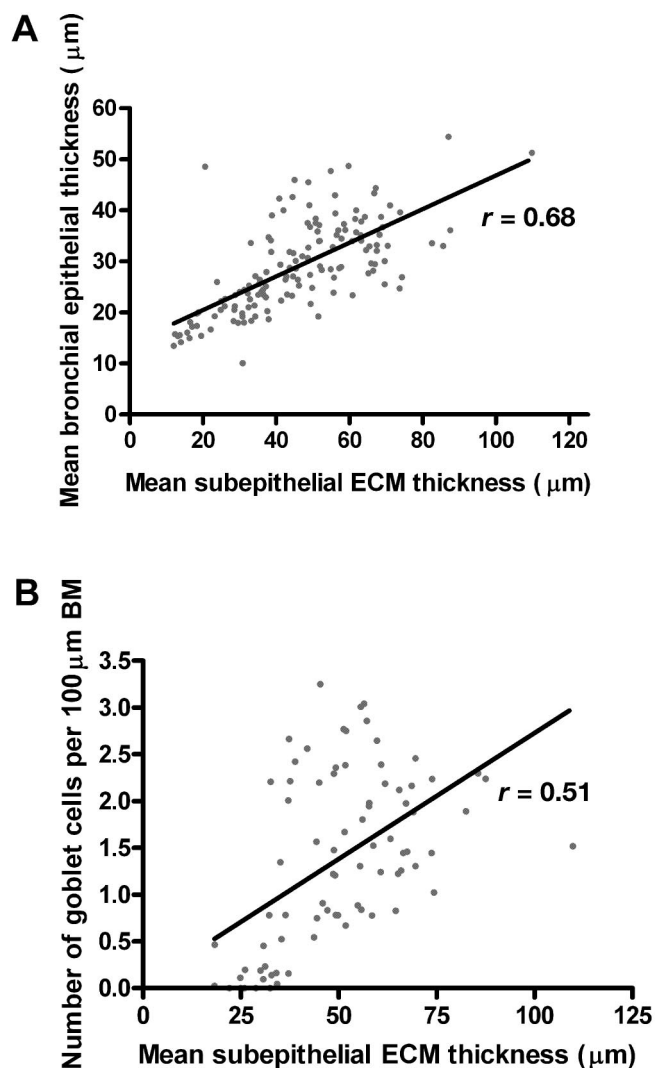


Figure 5. Pearson correlation analysis of mean airway wall thicknesses in a mouse model of chronic allergic airway disease. A, There was a moderately strong ($r = 0.68$, $P < .001$) correlation between mean epithelial thickness and mean subepithelial extracellular matrix (ECM) thickness ($n = 143$). B, There was a moderate ($r = 0.51$, $P < .001$) correlation between number of goblet cells and mean subepithelial ECM thickness ($n = 80$). BM indicates basement membrane.

supernatant fluid. TGF- β 1 has been shown to drive myofibroblast differentiation⁴⁴ and suppress myofibroblast apoptosis.⁴⁵ The main source of TGF- β 1 in asthma is the epithelium and eosinophils, and we found much lower levels of TGF- β 1 in fibroblasts than in epithelial cells in vitro. TGF- β 1 secreted from repairing epithelium⁴⁶ may facilitate migration of epithelial cells in the repair process.^{47,48} Increased TGF- β 1 production by BECs in asthma has been correlated with underlying fibroblast numbers and RBM thickness.⁴⁸

Abrogation of a key regulator of fibrosis, relaxin, in knockout mice resulted not only in airway fibrosis but also in other

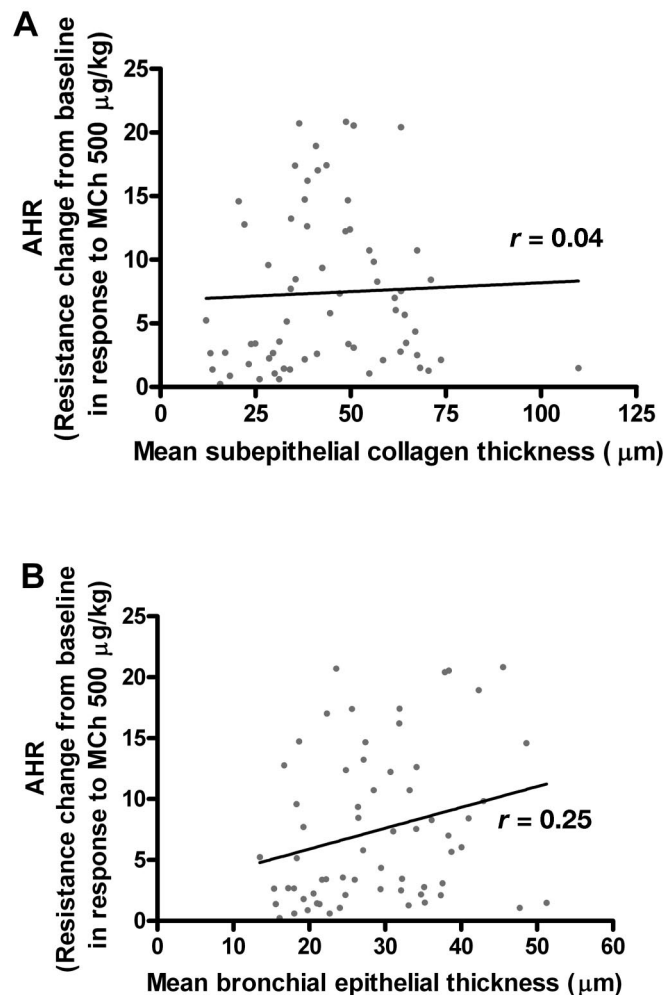


Figure 6. Pearson correlation analysis of mean airway wall thicknesses and airway hyperresponsiveness (AHR) in a mouse model of chronic allergic airway disease. A, There was little to no correlation between mean subepithelial extracellular matrix (ECM) thickness and AHR induced by methacholine (MCh), 500 $\mu\text{g/kg}$ ($r = 0.04$, $P = .74$) ($n = 64$). B, There was a weak correlation between mean epithelial thickness and AHR induced by MCh, 500 $\mu\text{g/kg}$ ($r = 0.25$, $P < .01$) ($n = 64$).

morphologic changes to the airway. In aged relaxin-deficient mice, the bronchial epithelium thickness was increased⁴¹ and further increased when a model of AAD was applied to these mice.⁴⁹

In the current study, we found a moderately strong correlation between subepithelial ECM deposition and epithelial thickness and a moderate correlation between subepithelial ECM deposition and goblet cell numbers. These results in many mice provide more evidence of the close association between fibrosis and epithelial changes, including thickening and goblet cell hyperplasia in AAD.

AHR is a functional outcome of asthma and a clinical marker of the disease. To our knowledge, no current therapies

directly target AHR.⁵⁰ In this study, AHR was shown to correlate weakly with mean bronchial epithelial thickness. In contrast, little to no correlation between AHR and subepithelial collagen deposition was observed. This finding suggests that the epithelium may contribute to remodeling changes to a greater extent compared with subepithelial fibrosis, therefore supporting the idea that epithelial remodeling events may be an important factor contributing to persistent AHR in patients optimally treated with inhaled corticosteroids.⁷ A proposed paradigm for the progression of airway remodeling might be that subepithelial collagen deposition may promote epithelial remodeling changes that exacerbate AHR. However, the relationship between individual remodeling changes and AHR remains controversial in asthma,^{51,52} but murine AAD and cell culture models may play an important role in solving this problem.⁵⁰

Although in vivo airway remodeling changes may progress over a lifetime, the experiments in the current in vitro study have been conducted during a 6-day culture period. The in vitro findings are of limited applicability to the more complex in vivo events in airway remodeling, in which other secreted factors regulate airway cell differentiation and proliferation. However, our results offer a starting point for further investigations of the role of ECM in airway remodeling of BECs, fibroblasts, and other cell types, including airway smooth muscle cells.^{53,54} Coculture of epithelial cells and fibroblasts has been useful in demonstrating other ways in which fibroblast secretions affect epithelial cell activation.

We have shown in vitro that abnormal collagen substrates can cause changes that have similarities with the pathology of airway remodeling. Thickening of the airway wall is caused in part by proliferation of fibroblasts in the lamina propria and by proliferation and differentiation of epithelial cells in goblet cell hyperplasia. Airway fibrosis may drive these changes and up-regulate itself via TGF- β 1 production independently of active inflammation.

Airway remodeling has been identified as an important target for future therapies for asthma.⁵⁵ A better understanding of the importance of ECM deposition may be important for new treatments. Given the evidence that fibrosis drives other airway remodeling changes, maintaining levels of ECM deposition at near basal levels and normal BM composition using antifibrotic drugs holds promise for asthma treatment strategies.

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