

Extracellular matrix proteins modulate asthmatic airway smooth muscle cell proliferation via an autocrine mechanism

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Background: Airway remodeling is a key feature of persistent asthma and includes alterations in the extracellular matrix protein profile around the airway smooth muscle (ASM) and hyperplasia of the ASM. We have previously shown that nonasthmatic ASM cells in culture produce a range of extracellular matrix protein proteins and that asthmatic ASM cells proliferate faster than cells from nonasthmatic patients. **Objective:** In this study, we compared the profile of extracellular matrix proteins produced by nonasthmatic and asthmatic ASM cells. We also examined the influence of these extracellular matrix protein proteins and conditioned medium derived from nonasthmatic or asthmatic ASM cells on the proliferation of nonasthmatic and asthmatic ASM cells.

Methods: Extracellular matrix proteins were measured by ELISA; proliferation of ASM cells was measured by tritiated thymidine incorporation.

Results: Production of perlecan and collagen I by the cells from asthmatic patients were significantly increased. In contrast, laminin $\alpha 1$ and collagen IV were decreased. Chondroitin sulfate was detectable only in the cells from nonasthmatic patients. Compared with nonasthmatic extracellular matrix proteins, proteins from asthmatic cells enhanced ASM cell proliferation. Conditioned medium from asthmatic ASM cells did not induce greater proliferation compared with conditioned medium from nonasthmatic cells.

Conclusions: The data show that the profile of extracellular matrix protein components is altered in asthmatic cells and that this altered profile and not soluble mediators secreted from the ASM cells has the potential to influence the proliferation of these cells. These changes are likely to contribute to the airway wall remodeling that occurs in asthma. (*J Allergy Clin Immunol* 2004;113:690-6.)

Key words: Remodeling, airway smooth muscle proliferation, extracellular matrix, asthma

Abbreviations used

ASM: Airway smooth muscle
CPM: Counts per minute
DMEM: Dulbecco modified Eagle medium
ECM: Extracellular matrix protein
HASM: Human airway smooth muscle
TdR: [³H]-thymidine

Tissue remodeling is a key feature of persistent asthma and includes an increase in the bulk of the airway smooth muscle (ASM). This increase is due in part to a combination of increased deposition of extracellular matrix (ECM) proteins around the ASM¹ and hyperplasia of the ASM.² These structural changes in the airways are thought to contribute to the persistent airway obstruction and bronchial hyperresponsiveness of the airways of asthmatic patients by adding to the increased airway wall thickness and the stiffness of the airways, thereby influencing the contractility/relaxation of the airway.^{3,4}

In histologic studies of asthmatic airways, the general profile of ECM proteins is altered. Collagen I, III, and V, fibronectin, tenascin, hyaluronan, versican, and laminin $\alpha 2/\beta 2$ are increased,⁵⁻⁸ whereas collagen IV and elastin are decreased.^{9,10} Specifically around the ASM of asthmatic patients, increases in collagen I,¹¹ hyaluronan, and versican⁷ have been observed. The intricate network of macromolecules forming the ECM not only acts as mechanical support that plays a crucial role in the maintenance of airway structure and function but also has the potential to influence a variety of cellular function, including proliferation.¹²

To date, the mechanism leading to the hyperplasia of ASM cells in the airways of asthmatic patients is unknown. Many studies have been performed examining the effect of various growth mediators on the proliferation of human ASM cells in culture.¹³ A variety of mediators implicated in asthma have been shown to induce proliferation of ASM; however, these studies have been performed on ASM cells obtained from nonasthmatic subjects. We have previously shown that ASM cells obtained from asthmatic patients proliferate at a greater rate than ASM cells from nonasthmatic patients.¹⁴

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TABLE I. Patient information

Patient no.	Age	Sex	Nonasthmatic/asthmatic	Type of sample	Atopic	Medication	FEV ₁ % predicted
1	52	Female	Nonasthmatic	Explant lungs	No	c	NA
2	NA	NA	Nonasthmatic	Explant lungs	No	n	NA
3	55	Male	Nonasthmatic	Explant lungs	NA	NA	NA
4	73	Female	Nonasthmatic	Resection	NA	NA	NA
5	76	Male	Nonasthmatic	Resection	NA	NA	NA
6	40	Male	Nonasthmatic	Autopsy	No	n	NA
7	47	Male	Nonasthmatic	Explant lungs	Yes	n	NA
8	38	Male	Nonasthmatic	Explant lungs	No	NA	NA
9	20	Male	Nonasthmatic	Endobronchial biopsy	No	n	87%
10	21	Female	Nonasthmatic	Endobronchial biopsy	No	n	90%
11	57	Male	Nonasthmatic	Explant lungs	No	NA	NA
12	52	Female	Nonasthmatic	Explant lungs	No	c	NA
13	61	Male	Nonasthmatic	Explant lungs	No	NA	NA
14	66	Male	Nonasthmatic	Resection	NA	n	60%
15	50	Male	Nonasthmatic	Explant lungs	Yes	NA	NA
16	65	Male	Nonasthmatic	Resection	No	NA	NA
17	76	Female	Nonasthmatic	Resection	NA	n	NA
18	25	Female	Nonasthmatic	Explant lungs	No	NA	NA
19	66	Male	Nonasthmatic	Resection	NA	n	NA
20	63	Female	Nonasthmatic	Resection	No	a	NA
21	16	Male	Nonasthmatic	Autopsy	No	n	NA
22	66	Male	Nonasthmatic	Resection	NA	n	NA
23	16	Female	Nonasthmatic	Resection	NA	n	NA
24	33	Male	Asthmatic	Explant lungs	Yes	b, c	76%
25	15	Male	Asthmatic	Autopsy	Yes	b, c	NA
26	37	Male	Asthmatic	Endobronchial biopsy	Yes	b, c	67%
27	44	Male	Asthmatic	Explant lungs	Yes	b	NA
28	80	Male	Asthmatic	Autopsy	Yes	b, c	NA
29	44	Female	Asthmatic	Endobronchial biopsy	Yes	b, c	109%
30	58	Male	Asthmatic	Endobronchial biopsy	Yes	b, c	92%
31	30	Female	Asthmatic	Endobronchial biopsy	Yes	a, b, c	64%
32	55	Female	Asthmatic	Endobronchial biopsy	Yes	b, c	97%
33	49	Female	Asthmatic	Endobronchial biopsy	Yes	b, c	84%
34	34	Female	Asthmatic	Endobronchial biopsy	Yes	b, c	79%
35	43	Female	Asthmatic	Endobronchial biopsy	Yes	b, c	85%
36	40	Male	Asthmatic	Endobronchial biopsy	Yes	b, c	47%
37	20	Female	Asthmatic	Endobronchial biopsy	Yes	b	75%

a, Anticholinergics; b, β -agonists; c, corticosteroids; n, no treatment; NA, information not available.

We hypothesized that remodeling in asthmatic airways could be associated with an altered profile of ECM proteins and enhanced proliferation of asthmatic ASM. We have previously reported that ASM cells obtained from nonasthmatic patients produce a variety of ECM proteins.¹⁵ In this study, we compared the production of 15 different ECM proteins by nonasthmatic and asthmatic ASM cells and examined the effect of conditioned medium from asthmatic ASM cells in culture on the proliferation of nonasthmatic ASM cells. Furthermore, we examined the effect of an ECM created from nonasthmatic and asthmatic ASM cells on the proliferation of both nonasthmatic and asthmatic ASM cells. The ECM proteins examined in this study were chosen to represent a cross-section of the ECM proteins likely to be found in the airways and to include basement membrane proteins, connective tissue proteins, adhesive glycoproteins, proteoglycans, and glycosaminoglycans.

METHODS

Cell culture

We obtained human ASM cells from 23 nonasthmatic (50 ± 20 years; mean $\pm$ SD) and 14 asthmatic (42 ± 16 years; mean $\pm$ SD) patients by methods adapted from those previously described.^{15,16} The characteristics of the patients are listed in Table I. Approval for all experiments with human lung was provided by the Human Ethics Committee of the University of Sydney and the Central Sydney Area Health Service. ASM cell characteristics were determined by immunofluorescence and light microscopy as previously described.¹⁷ All experiments were performed with cells between passages 3 and 8.

ECM protein experiments

ECM proteins were measured by ELISA, as previously described.¹⁸ ASM cells from 6 nonasthmatic and 6 asthmatic patients were seeded into 48 wells of 96-well plates in Dulbecco modified Eagle medium (DMEM) supplemented with 10% serum. Cells were grown for 7 days, with replenishment of all the conditions at day 4.

TABLE II. RT-PCR conditions

Gene	Primers	Annealing temperature	No. of cycles	Product size
18S rRNA	Forward primer (0.5 μ mol/L) 5' CTCAACACGGGAAACCTCAC 3' Reverse primer (0.5 μ mol/L) 5' GACAAATCGCTCCACCAACT 3'	56°C	12	112 bp
Collagen I	Forward primer (0.5 μ mol/L) 5' GAGACTGGCAACCTGAAAAA 3' Reverse primer (0.5 μ mol/L) 5' CCAAAGGTGCAATATCAAGG 3'	56°C	26	199 bp
Collagen IV	Forward primer (1 μ mol/L) 5' AAGCTGTAAGCGTTTGCCTA 3' Reverse primer (1 μ mol/L) 5' ACTCTTTTGTGATGCACACCA 3'	59°C	26	120 bp
Laminin	Forward primer (0.25 μ mol/L) 5' GCGAATCTTGTGTTCCCTGT 3' Reverse primer (0.25 μ mol/L) 5' TTGGTTTGCAGTCACAGAGC 3'	44°C	26	249 bp
Perlecan	Forward primer (0.5 μ mol/L) 5' GCACAGTGCCCCAGTTCCCAA 3' Reverse primer (0.5 μ mol/L) 5' TCCGTCGCAGAGGTAGTCTC 3'	50°C	26	339 bp
Versican	Forward primer (0.5 μ mol/L) 5' CATTATCGACGAAGATTGG 3' Reverse primer (0.5 μ mol/L) 5' CAGGGTTGACTTCTTGCCTA 3'	56°C	26	200 bp

ECM free of cells was prepared by treatment with sterile hypotonic ammonium hydroxide. ELISA assays were performed by using antibodies to the following matrix proteins: human fibronectin, perlecan, elastin, laminin- β 1, γ 1, β 2, and α 1 chain, thrombospondin, chondroitin sulfate, collagen types I, III, IV, and V, versican, and decorin.¹⁹ In separate 6-well plates, cells were seeded at the same density in 10% human serum in DMEM. Cell numbers from the 6-well plates were assessed by manual cell counts.

Reverse transcriptase–polymerase chain reaction

Total RNA was extracted from ASM cells from 6 nonasthmatic and 6 asthmatic patients at various time points (0, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 1 day, and 3 days), using the RNeasy Mini Kit (Qiagen, Clifton Hill, Victoria, Australia). RT-PCR was performed with the use of a Superscript 1-step RT-PCR kit with Platinum Taq (Invitrogen, Sydney NSW, Australia). For quantitative analysis of gene expression, 18S rRNA was also amplified from each sample. For each reaction, the master mix containing the RNA sample was divided in half, with half used for the specific gene reaction and the other half for the 18S rRNA reaction.

The primers used are described in Table II. For all reactions, the reverse transcriptase step was performed at 50°C for 30 minutes. The amplification reactions commenced with an initial denaturation step at 94°C for 2 minutes. This was followed by denaturation at 94°C for 30 seconds, annealing at the temperature indicated in Table II for 30 seconds, and extension at 72°C for 1 minute. The number of cycles is indicated in Table II. For all reactions, a final extension of 72°C for 10 minutes was performed.

The reaction products were separated on 10% TAE (40 mmol/L tris-acetate, 1 mmol/L EDTA) polyacrylamide gels by electrophoresis and stained with 0.2% silver nitrate solution. Bands were analyzed with the 440F Kodak imaging system and software. Amounts of mRNA are expressed as a ratio of 18S rRNA present in each sample.

Proliferation experiments

Conditioned medium experiments

Conditioned medium collection. Human airway smooth muscle (HASM) cells from 3 asthmatic and 3 nonasthmatic patients were seeded at a density of 1×10^4 cells/cm² in 175² cm² flasks in 5% FBS in DMEM. After 24 hours, medium was changed to 1% FBS in DMEM to equilibrate the cells. Cells remained in this solution for 24 hours before the medium was replaced with 5% FBS in DMEM. At the end of 3 days in the 5% FBS medium, conditioned medium was collected from each flask and stored at -70°C .

Conditioned medium and proliferation. HASM cells from 4 asthmatic and 3 nonasthmatic patients were seeded in 12-well plates in 5% FBS in DMEM for 24 hours before changing to 0.1% FBS in DMEM for a further 24 hours. Cells were then grown in conditioned medium obtained from the asthmatic and nonasthmatic patients. Proliferation was assessed by manual cell counting.

Reseed experiments

ECM free of cells from 4 nonasthmatic and 6 asthmatic was prepared as above in 96-well plates and frozen at -20°C . ECM plates were defrosted on the day of the experiment and washed 5 times in sterile PBS. ECM plates were then seeded with ASM cells (1×10^4 cells/cm²) from 4 nonasthmatic or 7 asthmatic patients in 0%, 1%, 5%, and 10% FBS or human serum. The degree of proliferation in the 96-well plates was assessed on days 1, 2, and 3 by measuring [³H]-thymidine (TdR) incorporation.

TdR incorporation. After incubation for 19, 43, or 67 hours in the various conditions being tested, 1 μCi TdR in 10 μL of DMEM was added to the 100 μL of medium in all wells and incubated for a further 5 hours at 37°C.²⁰ Cell proliferation was then arrested by freezing at -20°C . The plates were later defrosted, 100 μL of 1 mol/L NaOH added to each well, then harvested onto UniFilter 96 GF/C filter plates (Packard, Australia), using the Filtermate Unifilter-96 Harvester

(Packard, Australia). UniFilter plates were then dried overnight at room temperature before addition of 20 μ L of Microscint 0 scintillant (Packard, Australia) to each well. Counts per minute (CPM) were obtained on a TopCount Microplate scintillation counter (Packard, Australia) for 1 minute per sample.

Analysis of results

Results of the ECM ELISAs are expressed as a percentage of the total of the 15 proteins produced. Results from the mRNA experiments are expressed as a ratio of the densitometry units for the gene of interest and 18S rRNA. Results from the conditioned medium experiments cell number was expressed as a percentage of the number of cells obtained before conditioned medium was added. Reseed TdR experiments were expressed as a percentage of the CPM obtained from cells in 0% serum in the absence of an ECM bed. Significant differences were assessed by paired Student *t* test or ANOVA, using the Fisher protected least squares difference posttest and a significance level of $P < .05$. ANOVA factorial with the Fisher protected least squares difference posttest and a significance level of $P < .05$ was used to assess difference between curve data constructed from the addition of 0%, 1%, 5%, and 10% serum. All results presented are means $\pm$ SEMs.

RESULTS

ECM composition in ASM cells

After 7 days of growth in the presence of 10% serum, the nonasthmatic and asthmatic ASM cells produced varying amounts of the 15 ECM proteins examined. When nonasthmatic and asthmatic ASM cells were compared, a significant increase was observed in perlecan ($7.9\% \pm 1.5\%$ nonasthmatic and $12.0\% \pm 1.1\%$ asthmatic, $P < .05$, $n = 6$ and 6 , respectively, ANOVA factorial) and collagen type I ($3.0\% \pm 0.5\%$ nonasthmatic and $8.5\% \pm 0.9\%$ asthmatic, $P < .05$, $n = 6$ and 6 , respectively, ANOVA factorial) (Fig 1). In contrast, a significant decrease was observed in laminin $\alpha 1$ ($4.3\% \pm 0.6\%$ nonasthmatic and $2.2\% \pm 0.4\%$ asthmatic, $P < .05$, $n = 6$ and 6 , respectively, ANOVA factorial) and collagen IV ($6.1\% \pm 1.9\%$ nonasthmatic and $1.4\% \pm 0.6\%$ asthmatic, $P < .05$, $n = 6$ and 6 , respectively, ANOVA factorial) (Fig 1). Moreover, chondroitin sulfate was not detectable in the ECM of asthmatic ASM cells, whereas it was present in nonasthmatic ASM cells ($3.9\% \pm 1.1\%$, $P < .05$, $n = 6$ and 6 , respectively, ANOVA factorial) (Fig 1).

ECM mRNA detection in nonasthmatic and asthmatic ASM cells

mRNA was detected in the ASM cells obtained from both nonasthmatic and asthmatic patients for collagen I, collagen IV, laminin, and perlecan. No significant differences were observed at any of the time points (0, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 1 day, and 3 days) examined in collagen I, collagen IV, laminin, or perlecan when the production in the cells from nonasthmatic and asthmatic patients was compared (data not shown). Specific mRNA for chondroitin sulfate could not be measured because there is no gene that directly encodes this glycosaminoglycan.

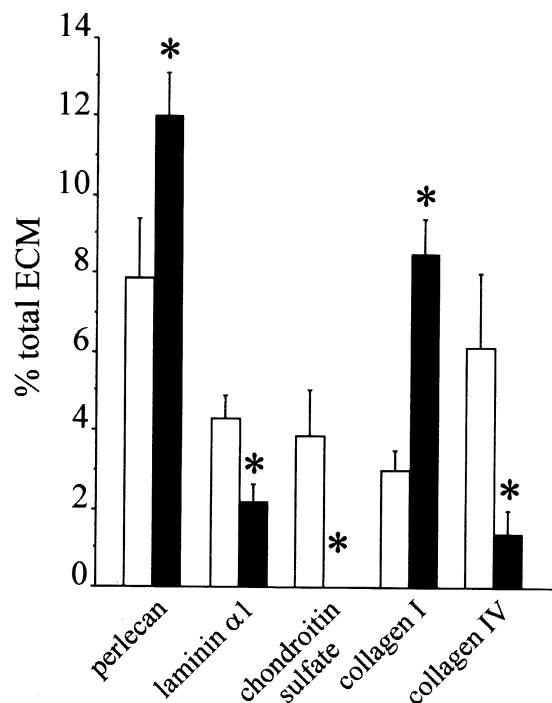


FIG 1. Production of ECM proteins by nonasthmatic (open bars) ($n = 6$) and asthmatic (closed bars) ($n = 6$) ASM cells as determined by ELISA. Results are expressed as a percentage of total ECM for the 15 different ECM proteins measured. *Significant difference from nonasthmatic, $P < .05$, ANOVA.

Conditioned medium experiments

When compared with conditioned medium from nonasthmatic ASM cells, the conditioned medium from the asthmatic cells had no additional mitogenic effect on the proliferation of either the nonasthmatic or asthmatic ASM cells over the 5 days examined (Fig 2, A and B). Proliferation of the asthmatic cells compared with the nonasthmatic cells was significantly greater at day 3 in the presence of asthmatic or nonasthmatic conditioned medium (Fig 2, A and B) ($126.6\% \pm 14.3\%$ nonasthmatic and $160.2\% \pm 14.2\%$ asthmatic, $P < .05$, $n = 3$ and 4 in nonasthmatic conditioned medium and $126.3\% \pm 18.3\%$ nonasthmatic and $150.0\% \pm 21.3\%$ asthmatic, $P < .05$, $n = 3$ and 4 in asthmatic conditioned medium, ANOVA).

Effect of ECM on proliferation

Asthmatic ECM induced enhanced proliferation of nonasthmatic cells in the presence of 5% ($348.2\% \pm 42.0\%$ nonasthmatic ECM and $551.7\% \pm 71.6\%$ asthmatic ECM, $P < .05$, $n = 13$) and 10% serum ($396.9\% \pm 32.2\%$ nonasthmatic ECM and $674.9\% \pm 153.3\%$ asthmatic ECM, $P < .05$, $n = 13$) (Fig 3, A). Proliferation of asthmatic cells was also enhanced by asthmatic ECM in the presence of 1% ($375.2\% \pm 51.6\%$ nonasthmatic ECM and $525.4\% \pm 84.5\%$ asthmatic ECM), 5% ($525.4\% \pm 84.5\%$ nonasthmatic ECM and $624.2\% \pm 60.8\%$ asthmatic ECM, $P < .05$, $n = 10$), and 10% ($442.1\% \pm 40.1\%$ nonasthmatic ECM and $510.8\% \pm 26.6\%$ asthmatic ECM, $P < .05$, $n = 10$) serum (Fig 3, B). Serum response

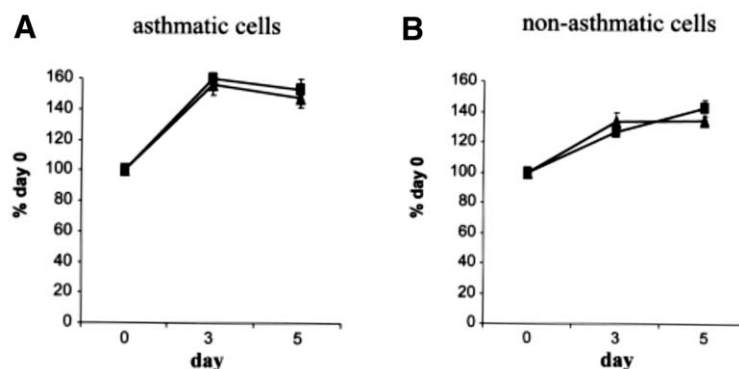


FIG 2. Effect of conditioned medium from asthmatic (boxes) (n = 3) and nonasthmatic (diamonds) (n = 4) ASM cells on proliferation of asthmatic (n = 3) and nonasthmatic (n = 3) ASM cells. Results are expressed as a percentage of cell counts obtained at day 0.

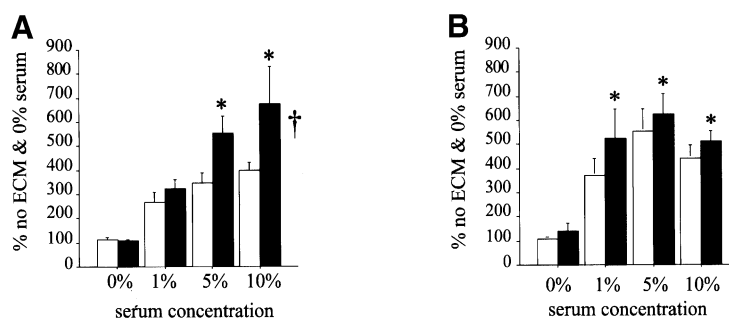


FIG 3. A, Effect of nonasthmatic (open bars) and asthmatic (closed bars) ECM on 5% serum induced proliferation of nonasthmatic ASM cells. **B,** Effect of nonasthmatic (open bars) and asthmatic (closed bars) ECM on 5% serum induced proliferation of asthmatic ASM cells. *Significant difference from nonasthmatic ECM, $P < .05$. †Significant difference from nonasthmatic curves, $P < .05$, ANOVA.

curves (0% to 10% serum) were significantly greater in nonasthmatic cells grown on the asthmatic ECM compared with nonasthmatic ECM ($P < .05$, $n = 13$, Fig 3, A).

DISCUSSION

In this study, we have shown that when ASM cells from asthmatic and nonasthmatic patients are compared, cells from asthmatic patients produce increased relative amounts of the structural protein collagen I, the basement membrane protein perlecan, and decreased amounts of the structural protein collagen IV, the glycosaminoglycan chondroitin sulfate, and the basement membrane protein laminin $\alpha 1$. These changes in ECM production are the result of posttranscriptional effects, as there were no differences in mRNA production between cells from asthmatic and nonasthmatic patients for collagen I, collagen IV, laminin, or perlecan. The enhanced proliferation of asthmatic ASM cells we have observed previously¹⁴ may in part be mediated by the influence of the ECM they produce and not by soluble autocrine factors as conditioned medium from asthmatic ASM cells failed to induce proliferation above that induced by conditioned medium from nonasthmatic cells.

The abnormalities in the ECM in the asthmatic airway can be localized external to the ASM, within the muscle

bundles,¹ or between the muscle and the epithelial layer. An increase in ECM deposition external to the smooth muscle may result in exaggerated airway narrowing through decreased airway-parenchymal interdependence.²¹ The impact of an altered ECM profile around the ASM in vivo may contribute to bronchial hyperresponsiveness by altering the stiffness of the airways, the contractility/relaxant capacity of the airways, and by adding to the increased airway wall thickness.^{3,4} Deposition of collagen,¹¹ hyaluronan, and versican⁷ in and around the ASM may result in opposition to smooth muscle contraction⁴; however, ECM deposition in this area would thicken the airway wall and exaggerate airway narrowing. It is also possible that an increase in ECM protein around the muscle may not necessarily result in increased stiffness, as would be expected, as the association between some ECM proteins may alter the structure/arrangement and formations of associated proteins. An example of this phenomenon is the interaction of collagen I with the proteoglycan perlecan, where this association may influence fibril assembly,²² resulting in the formation of thinner fibrils that in turn may have different mechanical properties compared with the normal thicker filament. Furthermore, proteoglycans, hyaluronan, and other members of the glycosaminoglycan family are

highly charged molecules that can strongly bind water. Binding of fluid in the airways has the potential to result in further thickening of the airway wall and a greater degree of airway narrowing to a given stimulus. Breakdown of the ECM associated with the ASM by the action of matrix metalloproteinases (MMPs) would decrease stiffness and the ASM would be effectively uncoupled from the load due to lung recoil, resulting in increased contraction.⁴ Subepithelial basement membrane ECM deposition may oppose the effects of smooth muscle shortening by increasing stiffness,²³ a concept that is supported by *in vivo* studies in which investigators have reported that distensibility of the airway wall was decreased in asthmatic patients.²⁴ The influence of the altered ECM profile in asthma is extremely difficult to assess on an individual ECM protein basis; however, there is no doubt that the altered profile has the potential to influence the contractile/relaxant properties of the airways and contribute to the asthmatic phenotype.

The results of the current study are in accordance with studies showing an increase in collagen I around the smooth muscle cells¹¹; however, the other ECM proteins observed to be changed in culture have not been studied *in vivo*, specifically in the region of the smooth muscle. Although perlecan has not been examined in the region of the smooth muscle in asthma, versican, another member of the proteoglycan family, has been shown to be increased in and around the muscle bundles.⁷ These two proteoglycans are likely to show similar water-binding profiles. Furthermore, studies in airways from asthmatic patients have shown that collagen IV is decreased in the airways. Although these studies did not examine specifically the region of the smooth muscle, they are in accordance with our findings of decreased collagen IV. Previously, the cells responsible for the production of this altered ECM profile around the ASM were unknown. This study provides evidence that the asthmatic ASM cell itself may be responsible for the altered profile in its own vicinity. The ECM proteins examined in this study were chosen to represent a cross-section of the ECM proteins likely to be found in the airways and to include basement membrane proteins, connective tissue proteins, adhesive glycoproteins, proteoglycans, and glycosaminoglycans.

It is unlikely that the differences observed between the asthmatic and nonasthmatic ASM cells reflect derivation from different sources, for example, endobronchial biopsy and resection material. The majority of the asthmatic ASM cells were obtained from endobronchial biopsy specimens, whereas the majority of the nonasthmatics were obtained from resected lung specimens. The size of the airway from which both cell types were taken were similar—the asthmatic cells from airways approximately 5-mm internal diameter and the nonasthmatic from airways approximately 4- to 6-mm internal diameter. Furthermore, in previous studies when two subsets of asthmatic ASM cells were compared—one derived from biopsy and the other from resection material—the type of specimen did not influence the outcome.¹⁴

The results from experiments examining matrix protein mRNA expression would suggest that the differences seen in ECM protein levels do not arise from altered control at the message level but are more likely to be the result of changes at a posttranscriptional level. Others have detected mRNA production for a variety of ECM proteins by nonasthmatic ASM, including collagen I, IV, fibronectin, decorin, and biglycan; however, in these studies, the amount of protein for the individual ECM proteins was not determined.²⁵

We have previously shown that ASM cells derived from asthmatic patients proliferate at a greater rate than cells derived from nonasthmatic patients.¹⁴ The mechanism for this enhanced proliferation is unknown; however, it may result from an autocrine effect of factors released from the ASM on muscle proliferation, as has been proposed in vascular smooth muscle cells.²⁶ These factors could be soluble factors released into the culture medium and could take the form of growth mediators or soluble ECM proteins. Other factors that could influence the proliferation of the ASM cells are the insoluble ECM proteins that are laid down by the muscle cells.²⁷ The results from the current study examining the effect of conditioned medium suggest that the enhanced proliferation of the asthmatic ASM in culture is not mediated by an autocrine soluble factor released from the ASM cells. These results do, however, suggest that the ECM deposited by the ASM cells has the potential to influence proliferation. The ECM in other systems potentially influences a number of cellular functions, including proliferation.^{12,28} Studies by Underwood et al¹² provide evidence that the ECM produced by vascular smooth muscle cells alters proliferation of human endothelial cells in culture. Furthermore, glycosaminoglycans are antiproliferative for pulmonary arterial smooth muscle cells.²⁸ In ASM cells, Hirst et al²⁹ showed that whereas fibronectin and collagen I enhanced proliferation, laminin caused a reduction. This study gives support to our findings of increased proliferation of the asthmatic ASM cells in the presence of enhanced collagen I and reduced laminin. Furthermore, the study by Garg et al²⁸ provides evidence for an antiproliferative effect of chondroitin sulfate; thus the relative lack of chondroitin sulfate in the asthmatic ECM may also lead to the enhanced proliferation of the cells on the asthmatic ECM.

In summary, we have shown that asthmatic ASM cells secrete increased relative amounts of perlecan and collagen I and decreased relative amounts of collagen IV, laminin $\alpha 1$, and chondroitin sulfate. These findings suggest that asthmatic ASM cells *in vivo*, by virtue of their production of an altered profile of ECM proteins, may contribute to the altered ECM around the ASM and thus contribute to remodeling in the airways.

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REFERENCES

- Bai TR, Cooper J, Koelmeyer T, Pare PD, Weir TD. The effect of age and duration of disease on airway structure in fatal asthma. *Am J Respir Crit Care Med* 2000;162:663-9.
- Ebina M, Takahashi T, Chiba T, Motomiya M. Cellular hypertrophy and hyperplasia of airway smooth muscle underlying bronchial asthma. *Am Rev Respir Dis* 1993;148:720-6.
- James AL, Pare PD, Hogg JC. The mechanics of airway narrowing in asthma. *Am Rev Respir Dis* 1989;139:242-6.
- Pare PD, Roberts CR, Bai TR, Wiggs BJ. The functional consequences of airway remodeling in asthma. *Monaldi Arch Chest Dis* 1997;52:589-96.
- Laitinen LA, Laitinen A, Altraja A, Virtanen I, Kampe M, Simonsson BG, et al. Bronchial biopsy findings in intermittent or "early" asthma. *J Allergy Clin Immunol* 1996;98:S3-S6.
- Laitinen A, Altraja A, Kampe M, Linden M, Virtanen I, Laitinen LA. Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid. *Am J Respir Crit Care Med* 1997;156:951-8.
- Roberts CR, Burke AK. Remodelling of the extracellular matrix in asthma: proteoglycan synthesis and degradation. *Can Respir J* 1998;5:48-50.
- Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;1:520-4.
- Bousquet J, Chanez P, Lacoste JY, White R, Vic P, Godard P, et al. Asthma: a disease remodeling the airways. *Allergy* 1992;47:3-11.
- Carroll N, Elliot J, Morton A, James A. The structure of large and small airways in nonfatal and fatal asthma. *Am Rev Respir Dis* 1993;147:405-10.
- Thompson RJ, Schellenberg RR. Increased amounts of airway smooth muscle does not account for excessive bronchoconstriction in asthma. *Can Respir J* 1998;5:61-2.
- Underwood P, Bean P, Whitelock J. Inhibition of endothelial cell adhesion and proliferation by extracellular matrix from vascular smooth muscle cells: role of type V collagen. *Atherosclerosis* 1998;141:141-52.
- Ammit AJ, Panettieri RA, Jr. Invited review: the circle of life: cell cycle regulation in airway smooth muscle. *J Appl Physiol* 2001;91:1431-7.
- Johnson PR, Roth M, Tamm M, Hughes M, Ge Q, King G, et al. Airway smooth muscle cell proliferation is increased in asthma. *Am J Respir Crit Care Med* 2001;164:474-7.
- Johnson P, Black J, Tamm M, Roth M. Effect of beclomethasone on expression of matrix metalloproteinases in human bronchial smooth muscle cells exposed to atopic asthmatic serum. [abstract]. *Am J Respir Crit Care Med* 2000;161:A442.
- Johnson PR, Roth M, Tamm M, Hughes M, Ge Q, King G, et al. Airway smooth muscle cell proliferation is increased in asthma. *Am J Respir Crit Care Med* 2001;164:474-7.
- Halayko AJ, Salari H, Ma X, Stephens NL. Markers of airway smooth muscle cell phenotype. *Am J Physiol* 1996;270:L1040-L1051.
- Johnson PR, Black JL, Carlin S, Ge Q, Underwood PA. The production of extracellular matrix proteins by human passively sensitized airway smooth-muscle cells in culture: the effect of beclomethasone. *Am J Respir Crit Care Med* 2000;162:2145-51.
- Underwood PA, Dalton BA, Steele JG, Bennett FA, Strike P. Anti-fibronectin antibodies that modify heparin binding and cell adhesion: evidence for a new cell binding site in the heparin binding region. *J Cell Sci* 1992;102:833-45.
- Johnson PR, Armour CL, Carey D, Black JL. Heparin and PGE2 inhibit DNA synthesis in human airway smooth muscle cells in culture. *Am J Physiol* 1995;269:L514-L519.
- Macklem PT. Theoretical basis of airway instability. Roger S. Mitchell Lecture. *Chest* 1995;107:87S-8S.
- Vogel KG, Paulsson M, Heinegard D. Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. *Biochem J* 1984;223:587-97.
- Roberts CR. Is asthma a fibrotic disease?. *Chest* 1995;107:111S-7S.
- Wilson JW, Li X, Pain MC. The lack of densitability of asthmatic airways. *Am Rev Respir Dis* 1993;148:806-9.
- Panettieri RA, Tan EM, Ciocca V, Luttmann MA, Leonard TB, Hay DW. Effects of LTD4 on human airway smooth muscle cell proliferation, matrix expression, and contraction in vitro: differential sensitivity to cysteinyl leukotriene receptor antagonists. *Am J Respir Cell Mol Biol* 1998;19:453-61.
- Shichiri M, Fukui N, Ozawa N, Iwasaki H, Hirata Y. Adrenomedullin is an autocrine/paracrine growth factor for rat vascular smooth muscle cells. *Regul Pept* 2003;112:167-73.
- Johnson PR, Black JL, Carlin S, Ge Q, Underwood PA. The production of extracellular matrix proteins by human passively sensitized airway smooth-muscle cells in culture: the effect of beclomethasone. *Am J Respir Crit Care Med* 2000;162:2145-51.
- Garg HG, Joseph PA, Thompson BT, Hales CA, Toida T, Imanari T, et al. Effect of fully sulfated glycosaminoglycans on pulmonary artery smooth muscle cell proliferation. *Arch Biochem Biophys* 1999;371:228-33.
- Hirst SJ, Twort CH, Lee TH. Differential effects of extracellular matrix proteins on human airway smooth muscle cell proliferation and phenotype. *Am J Respir Cell Mol Biol* 2000;23:335-44.