

ORIGINAL ARTICLE

AIRWAY DISEASES

Extracellular matrix in airway smooth muscle is associated with dynamics of airway function in asthma

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To cite this article: Yick CY, Ferreira DS, Annoni R, von der Thüsen JH, Kunst PW, Bel EH, Lutter R, Mauad T, Sterk PJ. Extracellular matrix in airway smooth muscle is associated with dynamics of airway function in asthma. *Allergy* 2012; DOI:10.1111/j.1398-9995.2011.02773.x

Keywords

asthma; extracellular matrix; respiratory function tests; smooth muscle.

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Accepted for publication 26 November 2011

DOI:10.1111/j.1398-9995.2011.02773.x

Edited by: Michael Wechsler

Abstract

Background: Altered deposition of extracellular matrix (ECM) in the airway smooth muscle (ASM) layer as observed in asthma may influence ASM mechanical properties. We hypothesized that ECM in ASM is associated with airway function in asthma. First, we investigated the difference in ECM expression in ASM between asthma and controls. Second, we examined whether ECM expression is associated with bronchoconstriction and bronchodilation *in vivo*.

Methods: Our cross-sectional study comprised 19 atopic mild asthma patients, 15 atopic and 12 nonatopic healthy subjects. Spirometry, methacholine responsiveness, deep-breath-induced bronchodilation (ΔR_{rs}) and bronchoscopy with endobronchial biopsies were performed. Positive staining of elastin, collagen I, III and IV, decorin, versican, fibronectin, laminin and tenascin in ASM was quantified as fractional area and mean density. Data were analysed using Pearson's or Spearman's correlation coefficient.

Results: Extracellular matrix expression in ASM was not different between asthma and controls. In asthmatics, fractional area and mean density of collagen I and III were correlated with methacholine dose-response slope and ΔR_{rs} , respectively ($r = 0.71$, $P < 0.01$; $r = 0.60$, $P = 0.02$). Furthermore, ASM collagen III and laminin in asthma were correlated with FEV₁ reversibility ($r = -0.65$, $P = 0.01$; $r = -0.54$, $P = 0.04$).

Conclusion: In asthma, ECM in ASM is related to the dynamics of airway function in the absence of differences in ECM expression between asthma and controls. This indicates that the ASM layer in its full composition is a major structural component in determining variable airways obstruction in asthma.

Airway remodelling in asthma includes alteration in extracellular matrix (ECM) deposition and increase in airway smooth muscle (ASM) mass (1, 2). It is difficult to associate aspects of remodelling with disease severity or degree of airways obstruction and hyper-responsiveness (3). However,

recent studies suggest that remodelling of ASM is an exception to this (4). First, it has been shown that the clinical expression of asthma (5), airway hyper-responsiveness (6) and impaired airway relaxation (7) are associated with mast cell counts in the ASM layer in asthma. Second, the deposition

Abbreviations

ASM, airway smooth muscle; ECM, extracellular matrix; ERS, European Respiratory Society; FEV₁, forced expiratory volume in 1 s; FOT, forced oscillation technique; FVC, forced vital capacity; GINA, Global Initiative for Asthma; H&E, haematoxylin and eosin; PC₂₀, provocative concentration of methacholine causing a 20% drop in forced expiratory volume in 1 s; PBS, phosphate-buffered saline; R_{rs} , respiratory system resistance; ΔR_{rs} , change in R_{rs} after deep inspiration; SMA, alpha-smooth muscle actin; X_{rs} , respiratory system reactance; ΔX_{rs} , change in X_{rs} after deep inspiration.

of ECM inside and outside the ASM layer in asthma seems to be related to its clinical severity and is altered as compared to healthy controls (8, 9).

Extracellular matrix affects both the synthetic proliferative (10–12) and mechanical properties of ASM, which may be reflected by the altered airway mechanics observed in asthma (13). However, contradictory results have been reported *in vitro* as to whether the ECM in the asthmatic ASM layer leads to enhanced or rather constrained shortening and force generation of the ASM cells. Elastic and collagen fibres in the airway wall may diminish bronchoconstriction by giving radial constraint to ASM or rather enhance it when peribronchial pressure becomes less negative because of airway remodelling (14, 15). Proteoglycans and glycoproteins, for instance versican and laminin, respectively, may play an important role as well in determining the resiliency of ASM (16, 17).

In a previous study performed in asthma patients only, we have demonstrated that FEV₁, airway hyper-responsiveness and deep-breath-induced bronchodilation were associated with the expression of selective ASM proteins and ECM components in endobronchial biopsies (18). It is unknown whether such structure–function relationship exists for major ECM components in ASM such as proteoglycans and glycoproteins. In particular, human *in vivo* studies regarding this structure–function relationship are lacking.

In this study, we hypothesized that ECM in the ASM layer is associated with airway function in asthma. The first aim was to investigate whether there are differences in ECM in the ASM layer between atopic asthma and healthy atopic and nonatopic controls. The second aim was to investigate whether there are significant associations between ECM in the ASM layer (elastin, collagen I, III and IV, the proteoglycans decorin and versican and the glycoproteins fibronectin, laminin and tenascin) and the dynamics of airway function *in vivo* (induced bronchoconstriction and bronchodilation).

Methods

Design and subjects

This cross-sectional study consisted of two visits. At visit 1, subjects were screened for eligibility to participate according to the inclusion and exclusion criteria. Spirometry and methacholine bronchoprovocation test were performed. At visit 2, the respiratory system resistance (R_{rs}) and reactance (X_{rs}) were measured followed by FEV₁ reversibility testing and bronchoscopy with endobronchial biopsies.

The study population consisted of three groups: (i) atopic mild asthma ($n = 19$); (ii) healthy atopic control ($n = 15$); (iii) healthy nonatopic control ($n = 12$). Subjects were recruited by the Department of Respiratory Medicine of the Academic Medical Centre Amsterdam.

Asthma patients had controlled disease according to GINA guidelines (19). The inclusion criteria were the following: aged 18–50 years; nonsmoking or stopped >12 months with ≤ 5 pack years; no exacerbations within 6 weeks prior to participation; steroid naïve or stopped using steroids by any dosing route ≥ 8 weeks prior to participation; no pulmonary

diseases other than asthma; no lung medication except inhaled short-acting β_2 -agonists as rescue therapy; airway hyper-responsiveness defined by a methacholine PC₂₀ ≤ 8 mg/ml; postbronchodilator FEV₁ >70% of predicted; atopy defined by a positive skin prick test. The inclusion criteria for healthy control subjects were similar to those of the asthma patients, except that they had no pulmonary diseases and no airway hyper-responsiveness (methacholine PC₂₀ >8 mg/ml). The atopic status of healthy controls was determined by skin prick test, to delineate the contribution of atopy as such to the ECM composition in the ASM layer.

All subjects gave written informed consent prior to enrolment. This study was approved by the Medical Ethics Committee of the Academic Medical Centre Amsterdam and is registered at the Netherlands Trial Register (NTR1306).

Airway function and skin prick test

Spirometry was performed according to European Respiratory Society (ERS) recommendations (20). Reversibility was determined by measuring FEV₁ pre- and postinhalation of 400 μ g salbutamol. PC₂₀ was measured by methacholine bronchoprovocation test using the standardized tidal volume method with a maximum methacholine dose of 16 mg/ml (21). Because 24 of the total 27 healthy control subjects did not reach a PC₂₀ at the highest methacholine concentration, we used the validated dose–response slope to analyse airway responsiveness (22). R_{rs} and X_{rs} were measured with a forced oscillation technique (FOT) device (Woolcock Institute, Sydney, Australia) at 8 Hz during 60 s of tidal breathing according to ERS recommendations (23). Additionally, deep-breath-induced bronchodilation was assessed by measuring the change in resistance (ΔR_{rs}) and reactance (ΔX_{rs}) after a deep inspiration (7). The skin prick test included 12 common aeroallergen extracts.

Bronchoscopy

Fibreoptic bronchoscopy was performed according to international recommendations (24). Participants received local anaesthetic by Lignocaine spray 1% in the nose, and 1% and 10% in the throat. Additional Lignocaine 1% solution was instilled into lung segments to dampen the cough reflex. The bronchial tree was inspected by autofluorescence bronchoscopy (SAFE 3000; Pentax, Tokyo, Japan). Two endobronchial biopsies per patient were taken at B⁴⁻⁶ of the right lung with a cup forceps (Pentax KW2411S). Special care was taken in positioning the forceps laterally to the bronchial carina to minimize the amount of connective tissue and at the same time to maximize the yield of ASM in the biopsies. After collection, the biopsy specimens were fixed in 4% buffered formaldehyde and embedded in paraffin.

Histochemistry and immunohistochemistry

Biopsy specimens were cut into 4- μ m sections and stained with H&E for initial analysis as described previously (7, 9). Next, sections were stained with Elastica-van Gieson for

analysis of elastin in the ASM layer. Antigen retrieval and the primary antibodies used for labelling of ECM (collagen I, III and IV, decorin, versican, fibronectin, laminin and tenascin) are shown in Table 1. Briefly, the paraffin sections were dewaxed and rehydrated. Prior to overnight incubation with the primary antibody, a 3% H₂O₂ solution was applied for 40 min to inhibit endogenous peroxidase activity. A streptavidin-biotin (LSAB kit; DAKO, Glostrup, Denmark) or a nonbiotin (Novocastra Novolink; Leica Biosystems, Newcastle Upon Tyne, UK) detection method was applied as secondary antibody staining. All sections were incubated with antibodies from the same batch within one session and counterstained with Harris haematoxylin. Negative control staining was performed by replacing the primary antibodies by phosphate-buffered saline (PBS) or isotype-matched control antibodies.

Morphometry

Images of the microscopic slides were captured digitally (Leica DMR; Leica Microsystems, Wetzlar, Germany). Selection of the ASM area for analysis was based on alpha-smooth muscle actin (SMA) stain and morphology of the ASM cells, which were elongated and disposed in bundles (18). The area of positive staining for each antibody within the selected ASM was determined by colour threshold. To define this threshold, sections of 6–8 subjects per study group stained with each antibody were analysed to achieve the best range of positivity. Afterwards, the colour data file created for each antibody was applied to all cases stained with the same antibody using Image-Pro Plus 4.1 (Media Cybernetics, Bethesda, USA) at 200 $\times$ magnification as described previously (9). Fractional area was expressed as percentage of the total selected ASM area. Mean density of the immunohistochemical staining was measured by the image analysis software.

Data analysis

Fractional area and mean density were compared between study groups using unpaired *t*-test or Mann–Whitney *U*-test. Correlations between ECM and airway function parameters were examined using Pearson's or Spearman's correlation coefficient. Statistical analyses were performed using SPSS 18 (IBM Corporation, Armonk, NY, USA) with a *P*-value of <0.05 considered statistically significant.

Statistical power was calculated from our previous data on smooth muscle elastin staining (8). This showed that 13 subjects per group allowed detecting 12% between-group difference in fractional area of elastin in ASM with at least an 80% power at the 5% level of significance.

Results

Subjects

The subject characteristics of the study groups were largely comparable (Table 2). As expected, postbronchodilator FEV₁% predicted was significantly lower, whereas the dose-response slope and *R*_{rs} were significantly higher in asthma as compared to healthy controls.

Fractional area and mean density of ECM in ASM

Biopsy specimens of 5 of 19 asthma patients, 3 of 15 healthy atopic and 2 of 12 healthy nonatopic subjects contained no ASM and therefore could not be analysed.

Figure 1 shows representative images of paraffin sections stained for elastin, collagen I, III and IV, decorin, versican, fibronectin, laminin and tenascin. The fractional area of elastin in ASM was not different between asthma and controls with or without atopy (*P* > 0.05, Fig. 1A). Similarly, there was no difference in fractional area or mean density of all other stained ECM components in ASM between the study groups (*P* > 0.05, Fig. 1B–I).

ECM in ASM and dynamics of airway function

There were highly significant correlations between fractional area and mean density of ECM in ASM and parameters reflecting bronchoconstriction or bronchodilation. Notably, this was observed in the asthma patients only. First, fractional area of collagen I was positively correlated with the methacholine dose-response slope (*r* = 0.71, *P* < 0.01, Fig. 2A). Second, there was a positive correlation between fractional area of collagen III and ΔR_{rs} (*r* = 0.60, *P* = 0.02, Fig. 2B). Finally, fractional area of collagen III and laminin was inversely correlated with FEV₁ reversibility (*r* = −0.65, *P* = 0.01, Fig. 2C; *r* = −0.54, *P* = 0.04, Fig. 2D). Similar significant correlations were observed when fractional area was replaced with mean density (Table 3). In contrast, there were no significant correlations between ECM in ASM and FEV₁ or other spirometric values representing the level of airway function (*P* > 0.05).

Table 1 Antibodies used for immunohistochemical analyses

Antibody	Pretreatment	Species	Dilution	Clone	Manufacturer
Collagen I	Citrate	Goat	1 : 2500	Polyclonal	US Biological, Swampscott, MA, USA
Collagen III	Trypsin	Mouse	1 : 400	III-53	Oncogene & Calbiochem, Darmstadt, Germany
Collagen IV	Citrate	Mouse	1 : 300	CIV-22	Dako, Glostrup, Denmark
Decorin	Chondroitinase	Rabbit	1 : 400	Polyclonal	Sigma-Aldrich, Saint Louis, MO, USA
Versican	Trypsin	Mouse	1 : 600	2-b-1	Seikagaku Co., Tokyo, Japan
Fibronectin	Citrate	Rabbit	1 : 15000	Polyclonal	Dako, Glostrup, Denmark
Laminin	Proteinase K	Mouse	1 : 500	Monoclonal	Sigma-Aldrich, Saint Louis, MO, USA
Tenascin	Pepsin	Mouse	1 : 12000	BC-24	Sigma-Aldrich, Saint Louis, MO, USA

Table 2 Characteristics of asthmatic and healthy subjects

	Asthma	Healthy	
		Atopic	Nonatopic
Subjects (<i>n</i>)	19	15	12
Men/Women (<i>n</i>)	4/15	5/10	2/10
Age (years)†	25 (21–47)	27 (20–49)	24 (20–29)
FEV ₁ postbronchodilator (%pred.)‡, *	96 (13)	109 (13)	109 (11)
FEV ₁ /FVC‡	0.81 (0.08)	0.89 (0.06)	0.85 (0.07)
PC ₂₀ (mg/ml)§	1.81 (0.95–3.46)	>8	>8
Dose–response slope (% decline)	3.05 (1.29–8.13)	0.32 (0.17–0.44)	0.17 (0.12–0.23)
FEV ₁ /μmol methacholine¶, **			
<i>R</i> _{rs} 8 Hz FOT (cmH ₂ O/l/s)‡, ***	3.68 (0.82)	2.99 (0.66)	3.05 (0.66)
<i>X</i> _{rs} 8 Hz FOT (cmH ₂ O/l/s)‡	0.35 (0.35)	0.35 (0.16)	0.31 (0.16)

FEV₁, forced expiratory volume in 1 s; FOT, forced oscillation technique; FVC, forced vital capacity; PC₂₀, provocative concentration of methacholine causing a 20% drop in forced expiratory volume in 1 s; *R*_{rs}, respiratory system resistance; *X*_{rs}, respiratory system reactance; % pred., % predicted.

†Mean (min–max).

‡Mean (SD).

§Geometric mean (95% CI).

¶Median (P25–P75).

*Asthma vs Healthy, atopic *P* = 0.008; Asthma vs Healthy, nonatopic *P* = 0.008.

**Asthma vs Healthy, atopic *P* < 0.001; Asthma vs Healthy, nonatopic *P* < 0.001.

***Asthma vs Healthy, atopic *P* = 0.012; Asthma vs Healthy, nonatopic *P* = 0.037.

Discussion

The present study demonstrates structure–function relationships between ECM within the ASM layer and indices of bronchoconstriction and bronchodilation in asthma. We observed that fractional area of collagen I and III was positively correlated with the methacholine dose–response slope and change in *R*_{rs} after deep inspiration, respectively. Additionally, fractional area of collagen III and laminin was inversely correlated with FEV₁ reversibility. These structure–function relationships existed in asthmatics only, even though there was no difference in fractional area and mean density of ECM between asthma and healthy controls. Extracellular matrix in the ASM layer was not associated with FEV₁ or other spirometric values representing the level of airway function. Our results indicate that the ECM in the ASM layer is related to the dynamics of airway function in asthma. This suggests that the ASM layer contributes to the physiological phenotype of asthma.

To our knowledge, this is the first *in vivo* human study examining the structure–function relationship between ECM in ASM and airway function using endobronchial biopsies in mild asthma patients and healthy controls. The association between ECM components and the dynamics rather than the level of airway function is a novel finding and contributes to the current evidence found in literature pointing towards the ASM layer as a key player in determining airway mechanics (13).

We did not observe differences in fractional area or mean density of ECM in ASM between asthma patients and healthy controls with or without atopy. Previous studies have shown that the extent of various aspects of airway remodel-

ling is different between asthma with varying severity (25, 26). Additionally, the elastic fibre content in the superficial elastic fibre network attached to the basement membrane is different between fatal asthma and healthy controls (27). When considering ECM in the ASM layer, fatal asthmatics showed increased fractional area of elastic fibre and fibronectin as compared to nonfatal asthmatics (9). These data suggest that ECM remodelling in ASM is dependent on disease severity. Therefore, it may not be surprising that the mild asthmatics in the current study did not show significantly different fractional area or mean density of ECM as compared to healthy controls. This is in keeping with an earlier observation showing that the percentage positive staining of decorin and versican inside the ASM layer of moderate asthma was not different from healthy controls (28).

We have analysed both the fractional area and mean density of several ECM components in ASM by immunohistochemistry. The quantitative results not only present a detailed analysis of the distribution of ECM components in ASM, but also give an estimate of the amount of ECM. Other morphometric analysis methods, for example immunoblotting, may have provided complementary data. However, previous studies have shown that immunohistochemistry data have a good correlation with results obtained by those analysis methods (29, 30).

Collagen I, III and laminin were significantly correlated with the dynamics of airway function. This was observed in asthma patients only, which fits in with the results of a previous study showing that airway wall mechanics are different in asthma as compared to controls (31). Yet, it is unclear whether ECM in ASM enhances or rather constrains shortening and force generation of the ASM cells. The results

of our study indicate that collagen I, III and laminin in the asthmatic ASM layer lead to a more reactive, stiff and less distensible airway as is shown by positive correlations with the dose-response slope and ΔR_{rs} , and inverse association

with FEV₁ reversibility. These results suggest that ECM in the ASM layer of asthma patients leads to a deterioration in airway function and enhanced airway narrowing. This is in keeping with the results from our previous study showing that airway hyper-responsiveness was inversely correlated with the ASM contractile proteins desmin and MLCK (18). Additionally, the results from our previous and current studies suggest that a decrease in ASM contractile proteins and an increase in ECM proteins lead to less change in ΔR_{rs} . This supports the hypothesis that in atopic mild asthma, allergen exposure may lead to a change in ASM phenotype from contractile to proliferative (18).

Extracellular matrix produced by ASM cells may not be the only component inside the ASM layer that determines airway function (32). It has been shown recently that ASM-derived ECM proteins stimulate mast cells to differentiate into a fibroblastoid phenotype, which may play an important role in causing airway dysfunction (33). Additionally, ASM phenotype itself may be influenced by ECM (16, 34) as well as intramuscular mast cells (5, 6, 35). Apparently, ASM cell phenotype and ASM microenvironment determine airway function in asthma, most likely in very close interaction.

The three groups of well-characterized subjects and the extensive physiology together with the quantitative immunohistochemistry seem to represent the strengths of our study. In addition, our study featured sufficient statistical power. However, there are some limitations. First, each study group had to consist of 13 subjects according to the *a priori* sample size calculation. However, only 12 subjects in the healthy atopic and 10 in the healthy nonatopic study group were available for statistical analysis. We renewed the power calculations by a new analysis based on the current numbers of subjects and found that a between-group difference of 13% in fractional area of elastin in ASM could be detected with a power of 80% at the 5% level of significance. We therefore believe that this study was sufficiently powered. Furthermore, as mentioned earlier, the absence of differences in fractional area of ECM components in ASM is in keeping with results from previous studies. Second, we have included mild asthma patients only, because patients had to have controlled disease without steroid therapy. The benefit is that this avoids any bias potentially induced by steroids. Third, biopsies were taken from subsegmental levels of the right lung. As the degree of airway remodelling in asthma may vary with asthma severity and location in the bronchial tree (9), subsequent studies are needed to allow a more detailed

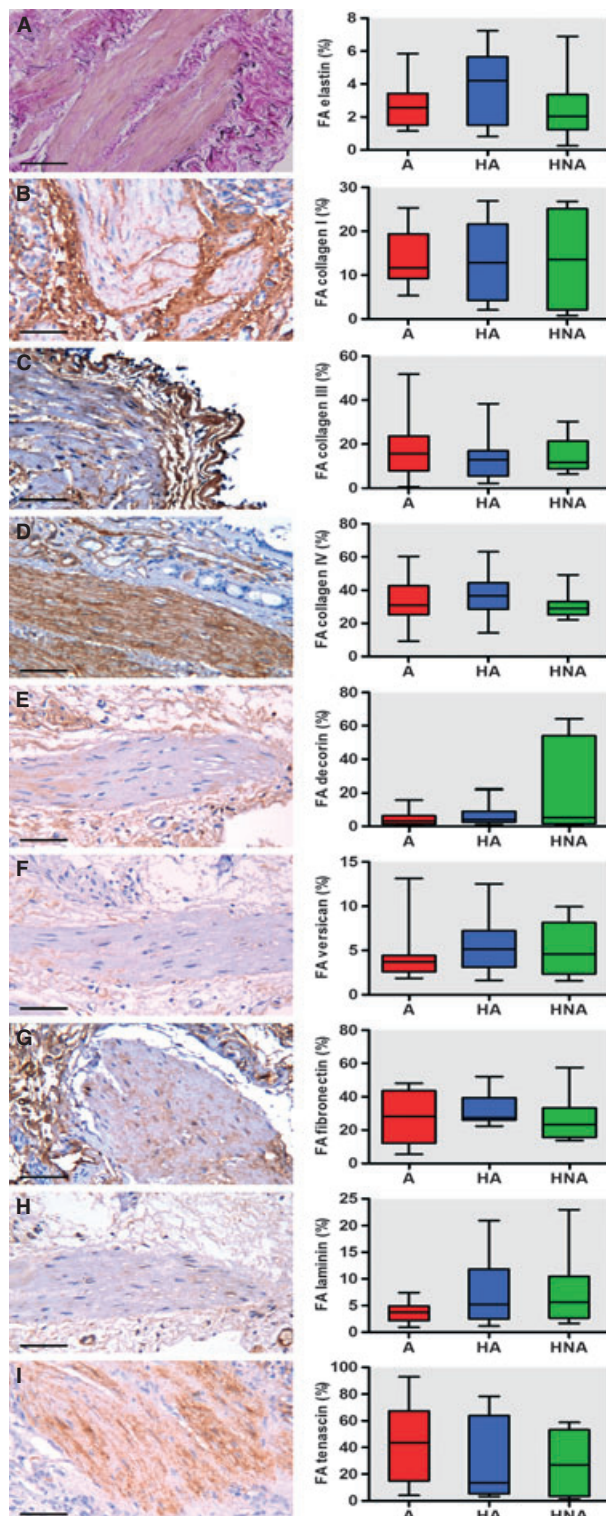


Figure 1 Extracellular matrix (ECM) inside the airway smooth muscle (ASM) layer. Representative images of biopsies stained for elastin (A), collagen I (B), III (C), and IV (D), decorin (E), versican (F), fibronectin (G), laminin (H), and tenascin (I). Fractional area of each ECM component is presented in a box-plot with the whiskers representing minimum and maximum values. Scale bar = 50 μ m; FA, fractional area expressed as percentage of the total selected ASM area per ECM component staining; A, asthma; HA, healthy atopic; HNA, healthy non-atopic.

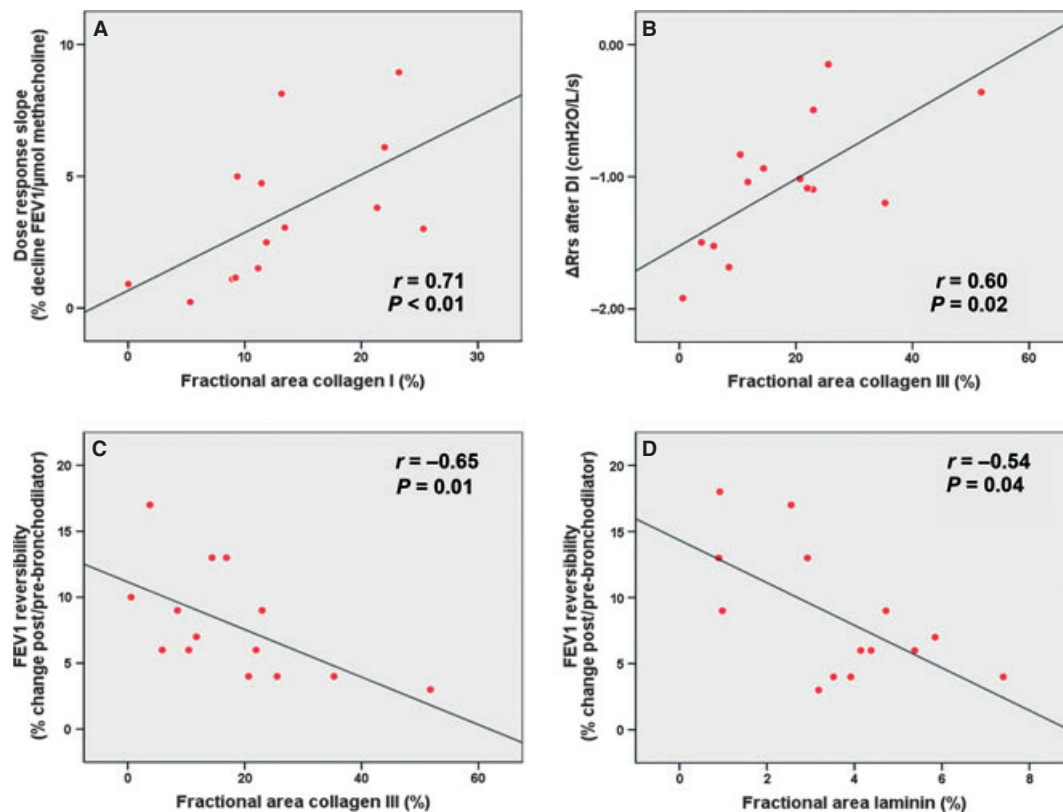


Figure 2 Correlations between extracellular matrix inside the airway smooth muscle layer and dynamics of airway function in patients with asthma. Significant correlations were found between fractional area of collagen I and dose response slope (A), collagen III and ΔR_{rs} (B), collagen III and FEV₁ reversibility (C), and laminin and FEV₁ reversibility (D). FEV₁, forced expiratory volume in 1 s; ΔR_{rs} after DI, change in respiratory system resistance after deep inspiration.

Table 3 Structure-function relationship in patients with asthma: fractional area and mean density

	Fractional area		Mean density	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
Collagen I – Dose–response slope	0.71	<0.01	0.81	<0.01
Collagen III – ΔR_{rs}	0.60	0.02	0.55	0.04
Collagen III – FEV ₁ reversibility	–0.65	0.01	–0.65	0.01
Laminin – FEV ₁ reversibility	–0.54	0.04	–0.54	0.04

FEV₁, forced expiratory volume in 1 s; ΔR_{rs} , change in respiratory system resistance after deep inspiration.

delineation of the structure–function relationship throughout the bronchial tree. Fourth, structural components of the airway wall other than the ASM layer, for instance the submucosa, may be important as well in determining airway function (36). Therefore, airway structural components and the inflammatory cell profile inside and outside the ASM layer need to be analysed in conjunction with airway physiology. In the future, this may result in the identification of

novel therapeutic targets leading to a more efficient, effective and personalized treatment, which not only relieves symptoms, but also tackles the pathologic basis of asthma at an early stage and thereby prevents deterioration of airway function.

Conclusions

This study shows structure–function relationships between ECM within the ASM layer and dynamics of airway function in patients with asthma. These findings were observed even in the absence of significant differences in fractional area or mean density of elastin, collagen I, III and IV, decorin, versican, fibronectin, laminin and tenascin in the ASM layer between asthma and healthy controls. Our study confirms and extends existing evidence showing that the ASM layer in its full composition is a major structural component of the airways in determining airway function in asthma.

Acknowledgments

This study was supported by a research grant from the Netherlands Asthma Foundation (project number 3.2.09.065). The Department of Pathology of the São Paulo University

Medical School (USP, Brazil) received support from the Brazilian Research Council (CNPq).

Authors contributions

CYY carried out the study procedures and spirometry measurements, participated in the design of the study and wrote the manuscript. DSF carried out the sectioning, immunohistochemical staining and analysis of the biopsy specimens and helped to draft the manuscript. RA carried out the sectioning, immunohistochemical staining and analysis of the biopsy specimens and helped to draft the manuscript. JHVD carried out the sectioning, histochemical staining and analysis of the biopsy specimens and helped to draft the manuscript. PWK performed all bronchoscopic proce-

dures and helped to draft the manuscript. EHB participated in the design of the study and its coordination and helped to draft the manuscript. RL participated in the design of the study and its coordination and helped to draft the manuscript. TM participated in the design of the study, coordinated the sectioning, immunohistochemical staining and analyses of the biopsy specimens and helped to draft the manuscript. PJS conceived the study, participated in the design of the study and its coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no competing interests.

References

- Fixman ED, Stewart A, Martin JG. Basic mechanisms of development of airway structural changes in asthma. *Eur Respir J* 2007;**29**:379–389.
- Ebina M, Takahashi T, Chiba T, Motomiya M. Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. A 3-D morphometric study. *Am Rev Respir Dis* 1993;**148**:720–726.
- Mauad T, Bel EH, Sterk PJ. Asthma therapy and airway remodelling. *J Allergy Clin Immunol* 2007;**120**:997–1009.
- James AL, Bai TR, Mauad T, Abramson MJ, Dolnikoff M, McKay KO et al. Airway smooth muscle thickness in asthma is related to severity but not duration of asthma. *Eur Respir J* 2009;**34**:1040–1045.
- Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002;**346**:1699–1705.
- Siddiqui S, Mistry V, Doe C, Roach K, Morgan A, Wardlaw A et al. Airway hyper-responsiveness is dissociated from airway structural remodeling. *J Allergy Clin Immunol* 2008;**122**:335–341.
- Slats AM, Janssen K, van Schadewijk A, van der Plas DT, Schot R, van den Aardweg JG et al. Bronchial inflammation and airway responses to deep inspiration in asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007;**176**:121–128.
- Araujo BB, Dolnikoff M, Silva LF, Elliot J, Lindeman JH, Ferreira DS et al. Extracellular matrix components and regulators in the airway smooth muscle in asthma. *Eur Respir J* 2008;**32**:61–69.
- Klagas I, Goulet S, Karakioulakis G, Zhong J, Baraket M, Black JL et al. Decreased hyaluronan in airway smooth muscle cells from patients with asthma and COPD. *Eur Respir J* 2009;**34**:616–628.
- Schuliga M, Ong SC, Soon L, Zal F, Harris T, Stewart AG. Airway smooth muscle remodels pericellular collagen fibrils: implications for proliferation. *Am J Physiol Lung Cell Mol Physiol* 2010;**298**:L584–L592.
- D'Antoni ML, Torregiani C, Ferraro P, Michoud MC, Mazer B, Martin JG et al. Effects of decorin and biglycan on human airway smooth muscle cell proliferation and apoptosis. *Am J Physiol Lung Cell Mol Physiol* 2008;**294**:L764–L771.
- Dekkers BG, Schaafsma D, Nelemans SA, Zaagsma J, Meurs H. Extracellular matrix proteins differentially regulates airway smooth muscle phenotype and function. *Am J Physiol Lung Cell Mol Physiol* 2007;**292**:L1405–L1413.
- An SS, Bai TR, Bates JH, Black JL, Brown RH, Brusasco V et al. Airway smooth muscle dynamics: a common pathway of airway obstruction in asthma. *Eur Respir J* 2007;**29**:834–860.
- Bramley AM, Roberts CR, Schellenberg RR. Collagenase increases shortening of human bronchial smooth muscle *in vitro*. *Am J Respir Crit Care Med* 1995;**152**:1513–1517.
- McParland BE, Macklem PT, Pare PD. Airway wall remodeling: friend or foe? *J Appl Physiol* 2003;**95**:426–434.
- Dekkers BGJ, Schaafsma D, Tran T, Zaagsma J, Meurs H. Insulin-induced laminin expression promotes a hypercontractile airway smooth muscle phenotype. *Am J Respir Cell Mol Biol* 2009;**41**:494–504.
- Iozzo RV. Matrix proteoglycans: from molecular design to cellular function. *Annu Rev Biochem* 1998;**67**:609–652.
- Slats AM, Janssen K, van Schadewijk A, van der Plas DT, Schot R, van den Aardweg JG et al. Expression of smooth muscle and extracellular matrix proteins in relation to airway function in asthma. *J Allergy Clin Immunol* 2008;**121**:1196–1202.
- GINA Report. Global strategy for asthma management and prevention (update December 2009). Available at: www.ginasthma.com.
- Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A et al. Standardisation of spirometry. *Eur Respir J* 2005;**26**:319–338.
- Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J* 1993;**16**(Suppl.):53–83.
- O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. *Am Rev Respir Dis* 1987;**136**:1412–1417.
- Oostveen E, MacLeod D, Lorino H, Farré R, Hantos Z, Desager K et al.; ERS Task Force on Respiratory Impedance Measurements. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J* 2003;**22**:1026–1041.
- Busse WW, Wanner A, Adams K, Reynolds HY, Castro M, Chowdhury B et al. Investigative bronchoprovocation and bronchoscopy in airway diseases. *Am J Respir Crit Care Med* 2005;**172**:807–816.
- Benayoun L, Druihe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003;**167**:1360–1368.

26. Pepe C, Foley S, Shannon J, Lemiere C, Olivenstein R, Ernst P et al. Differences in airway remodeling between subjects with severe and moderate asthma. *J Allergy Clin Immunol* 2005;**116**:544–549.
27. Mauad T, Xavier AC, Saldiva PH, Dolhnikoff M. Elastosis and fragmentation of fibers of the elastic system in fatal asthma. *Am J Respir Crit Care Med* 1999;**160**:968–975.
28. Pini L, Hamid Q, Shannon J, Lemelin L, Olivenstein R, Ernst P et al. Differences in proteoglycan deposition in the airways of moderate and severe asthmatics. *Eur Respir J* 2007;**29**:71–77.
29. Dittadi R, Catozzi L, Gion M, Brazzale A, Capitanio G, Gelli MC et al. Comparison between western blotting, immunohistochemical and ELISA assay for p185neu quantitation in breast cancer specimens. *Anticancer Res* 1993;**13**:1821–1824.
30. Ishikawa M, Mizukami K, Iwakiri M, Hidaka S, Asada T. Immunohistochemical and immunoblot study of GABA(A) alpha1 and beta2/3 subunits in the prefrontal cortex of subjects with schizophrenia and bipolar disorder. *Neurosci Res* 2004;**50**:77–84.
31. Brackel HJ, Pedersen OF, Mulder PG, Overbeek SE, Kerrebijn KF, Bogaard JM. Central airways behave more stiffly during forced expiration in patients with asthma. *Am J Respir Crit Care Med* 2000;**162**:896–904.
32. 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 beclomethason. *Am J Respir Crit Care Med* 2000;**162**:2145–2151.
33. Kaur D, Saunders R, Hollins F, Woodman L, Doe C, Siddiqui S et al. Mast cell fibroblastoid differentiation mediated by airway smooth muscle in asthma. *J Immunol* 2010;**185**:6105–6114.
34. Burgess JK, Ceresa C, Johnson SR, Kanabar V, Moir LM, Nguyen TT et al. Tissue and matrix influences on airway smooth muscle function. *Pulm Pharmacol Ther* 2009;**22**:379–387.
35. Alkhouri H, Hollins F, Moir LM, Brightling CE, Armour CL, Hughes JM. Human lung mast cells modulate the functions of airway smooth muscle cells in asthma. *Allergy* 2011;**66**:1231–1241.
36. Yick CY, von der Thüsen JH, Bel EH, Sterk PJ, Kunst PW. *In vivo* imaging of the airway wall in asthma: Fibered confocal fluorescence microscopy in relation to histology and lung function. *Respir Res* 2011;**12**:85.