

Bronchial matrix and inflammation respond to inhaled steroids despite ongoing allergen exposure in asthma

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Summary

Background Inflammatory and structural changes of the airway mucosa are chronic features of asthma. The mechanisms underlying these changes and their modulation by steroid prophylaxis have not been clarified.

Objective We postulated that asymptomatic ongoing allergen exposure could drive airway inflammation as well as changes in the extracellular matrix (ECM), and that inhaled steroids could prevent this.

Methods Therefore, we exposed patients with mild asthma to 2 weeks of repeated low-dose allergen, with concomitant inhaled steroid or placebo treatment. Bronchial biopsies, which were taken before and after this exposure, were stained and digitally analysed. The ECM proteins in asthmatics were also compared with a normal control group.

Results Low-dose allergen exposure alone resulted in a significant increase of bronchial epithelial macrophages. Despite ongoing allergen exposure, inhaled steroids reduced the numbers of mucosal eosinophils, neutrophils and T lymphocytes. At baseline, the mean density of the proteoglycans (PGS) biglycan and decorin were, respectively, higher and lower in the bronchial mucosa of asthmatics as compared with normal controls. Steroid treatment, during allergen exposure, increased the mean density of the PGS biglycan and versican.

Conclusion We conclude that chronic allergen exposure induces inflammatory changes in the bronchial mucosa. Despite ongoing allergen exposure, steroid treatment decreases mucosal inflammatory cells while altering PG density. The latter observation highlights the need to examine steroid-induced changes closely in the airway structure in patients with asthma.

Keywords allergens, asthma, bronchial biopsy, decorin, extracellular matrix, inflammation, proteoglycans, remodelling steroids, versican

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Introduction

Asthma is a chronic inflammatory disease, which persists in the majority of patients [1–3]. Next to airway inflammation, structural changes of the airways may lead to the development and chronicity of airway hyper-responsiveness (AHR) and eventually to an accelerated decline in lung function in asthmatics [2, 3]. The mechanisms resulting in persistent airway inflammation and structural changes have not been fully clarified. Chronic allergen exposure has been considered as a determinant of the maintenance of the disease and the accompanying inflammation [4]. However, the specific effects of such chronic exposure on the inflammatory and structural changes in the bronchial mucosa of asthmatics, and the possible modulation by inhaled steroids, are still unknown.

The patient's natural exposure to environmental allergens can be mimicked using an experimental human *in vivo* model with repeated low-dose allergen exposure. Such exposure has been shown to increase airway hyper-responsiveness [5–7], eosinophilic inflammation in sputum and bronchoalveolar lavage (BAL) [6–9] and a shift in the T helper (Th)2/Th1 cell balance [6, 9]. In addition to functional and inflammatory changes, animal models have demonstrated structural changes of the airways following repeated allergen exposure, such as an increase in goblet cell hyperplasia [10, 11], enhanced fibronectin [10, 11], and collagen deposition [10]. Interestingly, airway dysfunction and remodelling in mice persist even after resolution of acute allergen-induced airway inflammation [12]. These features of so-called 'airway remodelling' resemble those observed in patients with asthma [13].

Part of the remodelling process in asthma involves thickening of the reticular basement membrane [13] with altered deposition of extracellular matrix (ECM) proteins within the airways [14]. The ECM, which consists of e.g.

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collagen, fibronectin and proteoglycans (PGs), determines the biomechanical properties of the lung and influences the activity of cells that adhere to or migrate through the ECM [15]. Both fibronectin and PGs have been shown to be deposited early in processes of remodelling [10, 16], while collagen deposition only occurs after at least 12 weeks of chronic allergen exposure in animals [10]. PGs are involved in all aspects of ECM biology, including the determination of tissue mechanics, regulation of the water balance, cell adhesion, migration and proliferation, and biological activities of matrix-bound growth factors and cytokines [15]. Among the PGs in the ECM, biglycan, decorin and lumican belong to the family of small leucine-rich proteoglycans (SLRPs), whereas versican is a large PG and a member of the hyalectan gene family [17]. Thus far, there have been only a few papers focusing on PGs in biopsies from asthmatic patients [18–22]. Huang et al. [20] showed that patients with asthma have a larger area of positive staining in the airway mucosa for the PGs biglycan, lumican, and versican as compared with normal controls. However, there are no quantitative data regarding the deposition of the SLRP decorin, which has been shown to be antifibrotic by suppressing the bioactivity of transforming growth factor- β (TGF- β) [23, 24], in the airways of asthmatics and normals.

At present, inhaled steroids are the most effective therapy to control the clinical symptoms of asthma [1]. However, it appears that airway inflammation and structural changes are often not effectively suppressed during regular therapy in asthma, even when clinical symptoms are adequately controlled [25–27]. Inhaled steroid usage during repeated low-dose allergen exposure in asthmatics protects against the worsening of AHR and airway inflammation, as assessed in sputum [8, 9]. In animal models, concomitant treatment with steroids even inhibits the structural airway changes induced by repeated allergen exposure [11].

We postulated that structural changes and inflammation in the bronchial mucosa of patients with asthma are aggravated by ongoing allergen exposure, which can be inhibited by inhaled steroid treatment. Our aim was to test this hypothesis by exposing patients with mild asthma to 2 weeks of repeated

low-dose allergen inhalations, with and without inhaled steroid treatment.

Methods

Subjects

The study included 26 non-smoking, atopic subjects with mild asthma. Baseline forced expiratory volume in one second (FEV₁) was more than 75% of predicted and the PC₂₀ to methacholine was between 0.1 and 4 mg/mL. The patients were all dual responders to inhaled house dust mite (HDM) allergen, determined during a screening high-dose allergen challenge as published previously [9]. The patients were clinically stable, used β_2 -agonists on demand only, and had no history of a recent (≤ 2 weeks) upper respiratory tract infection or other relevant diseases. As little is known about the difference in content of ECM proteins between normal subjects and patients with asthma, we used biopsy sections of 12 non-atopic non-asthmatic subjects as controls that were available from a previously published study [28]. The Medical Ethics Committee of the Leiden University Medical Center approved the study, and the subjects gave their written informed consent before entering.

Study design

This pathology study was part of a large clinical trial [9]. The trial had a randomized, placebo-controlled, double-blind, parallel, two-arm design. The 26 patients were randomly assigned to either low-dose allergen exposure with concomitant inhaled steroid therapy ($n = 13$), or low-dose allergen and placebo treatment ($n = 13$). During the study, low-dose allergen inhalation was performed five times a week during 2 consecutive weeks starting at day 1. Thereafter, a 1-week washout period followed. Before (day-4) and after (day 15) the 2 weeks of allergen exposure, a bronchoscopy was performed and bronchial biopsies were taken. Double-blind, placebo-controlled treatment with 400 μ g inhaled budesonide

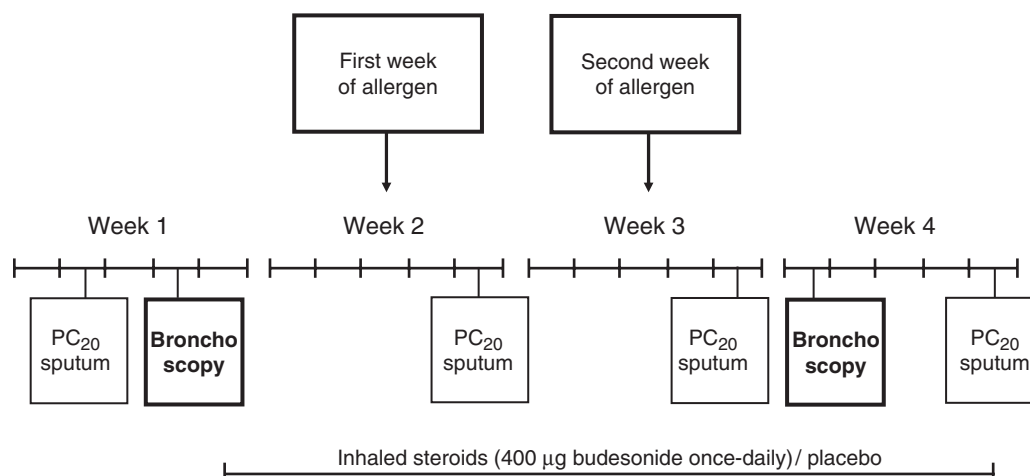


Fig 1. Study design in which the 4 study weeks are depicted by 5 working days (Monday–Friday). Low-dose allergen inhalation was performed in weeks 2 and 3 (days 1–5 and 8–12). Before and after these two weeks bronchoscopy was performed (days -4 and 15). Treatment with inhaled budesonide or placebo was administered from day -3 until day 19.

Table 1. Antibodies used for immunohistochemistry

Ab	Pretreatment	Species*	Dilution/conc.	Origin†
<i>Cell-specific markers</i>				
CD3 (lymphocyte)	Citrate	Ra	0.5 µL/mL	Dako
CD4 (T helper)	EDTA	M	1 : 50	Novocastra
CD8 (T cytotoxic)	EDTA	M	1 : 800	Novocastra
EG2 (eosinophil)	Trypsin	M	1 : 600	Pharmacia
Neutrophil elastase (neutrophil)	None	M	350 pg/mL	Dako
Tryptase AA1 (mast cell)	Citrate	M	6.56 pg/mL	Dako
CD68 (macrophage)	Citrate	M	30 pg/mL	Dako
<i>Markers for structural changes</i>				
Fibronectin	None	M	1 : 100	Novocastra
Biglycan	Chondroitinase-ABC	Ra	250 pg/mL	Ref‡
Decorin	Chondroitinase-ABC	Ra	4000 pg/mL	Ref‡
Lumican	Chondroitinase-ABC	Ra	500 pg/mL	Ref‡
Versican	Chondroitinase-ABC	M	500 pg/mL	Seikagaku

*M, mouse (monoclonal antibodies); Ra, rabbit (polyclonal antibodies); EDTA, ethylenediaminetetra-acetic acid.

†Dako, Glostrup, Denmark, Novocastra, Newcastle upon Tyne, UK, Pharmacia Diagnostics, Uppsala, Sweden. Seikagaku corporation/Brunschwig Chemie, Amsterdam, the Netherlands.

‡See ref 50 and 51

once daily was given from day -3 until day 19, by morning dose (Fig. 1).

Repeated low-dose allergen inhalations

The same purified HDM extract was used for screening high- and low-dose challenges (*Dermatophagoides pteronyssinus*, SQ 503; ALK Abelló, Nieuwegein, the Netherlands). The non-cumulative dose of allergen causing a fall in FEV₁ of 5% from post-diluent baseline during the screening allergen challenge was selected as the low dose to be used for subsequent repeated allergen exposure. The patients inhaled this as a single dose for 2 min at a fixed schedule on Monday, Tuesday, Wednesday, Thursday and Friday for 2 consecutive weeks [9].

Bronchoscopy and biopsy processing

Fibreoptic bronchoscopy was performed according to a standardized and validated protocol, which has been described previously [29]. Six biopsy specimens were taken at the (sub)segmental level, of which three were immediately fixed in phosphate-buffered saline (PBS) buffered formalin 10% (v/v) [29].

Biopsy staining

Sections of two formalin-fixed, paraffin-embedded biopsies with the best morphological quality per bronchoscopy were analyzed. Haematoxylin-eosin staining was used for evaluation of biopsy quality, and measurements of the reticular basement membrane. PAS/Alcian blue staining was performed to quantify the number of goblet cells in the bronchial epithelium. Immunohistochemical staining on paraffin-embedded tissue was performed on 3 µm thick biopsy sections (Table 1). If required, immunohistochemical staining was preceded by antigen retrieval. The sections, which were stained for the PGS, were incubated with 0.05 U/mL chondroitinase ABC (Sigma, Oakville, ON, Canada) for 1 h

at 37 °C. In short, the sections were incubated with an optimal dilution of antibodies in 1% bovine serum albumin (BSA)/PBS at room temperature for 60 min or overnight (CD4). As a secondary antibody the horseradish peroxidase-conjugated anti-mouse or anti-rabbit EnVision system (DAKO, Glostrup, Denmark) was used, with NovaRED (Vector, Burlingame, CA, USA) as the chromogen. The sections were counterstained with Mayer's haematoxylin [28]. For negative controls, the primary antibody was omitted from this procedure. Biopsy sections of the normal control group were stained and analysed simultaneously as the biopsies of the asthmatics for the ECM proteins, goblet cells and reticular basement membrane.

Analysis of bronchial biopsies

All biopsy specimens were coded before analysis. Measurements of the thickness of the reticular basement membrane and the number of positive epithelial cells for PAS/Alcian blue were performed interactively using the Zeiss KS400 system. The thickness of the reticular basement membrane was measured, using a light microscope, on haematoxylin-eosin-stained sections by taking 50 measurements at 20 µm intervals in areas of good epithelial orientation, i.e., in non-tangentially cut epithelium [27].

Fully automated densitometry and inflammatory cell counting procedures were developed using the Zeiss Vision KS400 system (Carl Zeiss, Göttingen, Germany) [29–31]. If possible, two digital images from every stained section (making a total of four images, which is the same as 16 20 × magnifications, per bronchoscopy per person) were obtained using a three-chip colour camera (1.732 × 10⁶ pixels; 1320 × 992 µm²; 3 × 256 greyvalues). Lamina propria was defined by the widest possible zone of maximal 125 µm deep beneath the reticular basement membrane, excluding broncho-alveolar lymphoid tissue, airway smooth muscle and damaged tissue [29, 30]. Likewise, the epithelial area was defined by the area above the reticular basement membrane

enclosed by the airway lumen not excluding damaged epithelium. Automated cell counts were performed in the epithelium and in the lamina propria by a validated method [30]. Positively stained cells were expressed as the number of cells/0.1 mm². Densitometric analyses of fibronectin and PG_S in the lamina propria (including the reticular basement membrane) were also carried out fully automated, by using a linear combination of Red-, and Blue-filtered greyscale images in order to derive a greyscale image (range 0–255) in which the 'brown-red' staining of interest is highlighted above a uniform background. This resulted in a narrow and peaked greyvalue distribution of background pixels with a longer tail on the left, which represents positive staining pixels. Fibronectin and PG_S staining intensity was expressed as the average greyvalue, after normalization of the distribution towards the background peak (white: greyvalue 255) and subsequent inversion of the greyvalue distribution [31].

Statistical analysis

The sample size of 13 patients per group in this study was based on our repeatability data with regard to immunohistochemistry in bronchial biopsies [29], allowing the detection of two-fold differences in bronchial biopsy cell numbers within and between groups, if $\alpha = 0.05$ and $1 - \beta = 0.80$. Weighted means for cell counts and densitometric analysis data were used. Cell counts were log transformed before analysis. All within-group changes were explored with two-tailed paired *t*-tests, and between-group differences were determined with two-tailed unpaired *t*-tests. *P*-values ≤ 0.05 were considered to denote statistically significant differences.

Results

All 26 patients completed the bronchoscopies of the study, except one subject of the steroid-treated group, who did not undergo the second bronchoscopy because of personal reasons.

Inflammatory cells

Epithelium (Table 2) First, in the placebo group we observed a significant increase in CD68⁺ cells after two weeks of low-dose allergen exposure (Day 15 as compared with day –4, $P = 0.05$). Moreover, after allergen exposure (Day 15), the number of CD68⁺ cells was significantly higher in the placebo group as compared with the steroid group ($P = 0.02$), while the changes during the study were also significantly different between both groups ($P = 0.02$). Second, there was a significant decrease in the number of elastase⁺ cells in the steroid group (day 15 as compared with day –4, $P = 0.03$). There were no significant changes in the numbers of other cells in the epithelium as a consequence of allergen exposure or steroid treatment.

Lamina propria (Table 3) Low-dose allergen exposure resulted in a non-significant increase in EG2⁺ cells in the placebo group (Day 15 as compared with day –4, $P = 0.09$). A significant decrease was observed in both the numbers of EG2⁺ and CD3⁺ cells in the steroid group (Day 15 as compared with day –4, $P = 0.002$ and $P < 0.001$, respectively), while there was a trend towards a significant decrease in the number of CD4⁺ cells ($P = 0.07$). Moreover, the

Table 2. Cell counts in the epithelium

Marker	Group	Pre-allergen day –4 (mean* [range])	Post-allergen day 15 (mean [range])	Paired <i>t</i> -test† <i>P</i>	Unpaired <i>t</i> -test Δ‡ <i>P</i>
EG2	PLAC	4.06 [0–20.6]	5.61 [0–16.9]	0.61	
	BUD	4.75 [0–17.9]	3.54 [0–47.9]	0.36	
PG _S		0.70	0.32		0.30
Elastase	PLAC	93.45 [11.1–482.0]	133.17 [7.2–395.6]	0.41	
	BUD	135.40 [24.7–543.4]	67.75 [29.8–173.8]	0.03	
<i>P</i>		0.40	0.08		0.06
AA1	PLAC	8.66 [1.9–38.2]	7.60 [0–23.3]	0.44	
	BUD	6.06 [0–23.1]	4.92 [0–18.1]	0.59	
<i>P</i>		0.33	0.27		0.86
CD68	PLAC	24.96 [6.1–129.0]	46.96 [14.0–182.9]	0.05	
	BUD	35.04 [13.6–71.9]	25.67 [7.0–56.3]	0.21	
<i>P</i>		0.22	0.02		0.02
CD3	PLAC	156.49 [27.2–384.8]	120.61 [39.8–319.5]	0.41	
	BUD	115.37 [63.2–274.3]	113.08 [65.2–207.1]	0.83	
<i>P</i>		0.23	0.80		0.41
CD4	PLAC	66.10 [5.7–257.7]	53.44 [11.7–508.9]	0.65	
	BUD	73.23 [5.3–339.5]	78.76 [10.5–438.2]	0.89	
<i>P</i>		0.81	0.41		0.65
CD8	PLAC	18.20 [4.3–36.8]	20.8 [3.2–102.1]	0.69	
	BUD	13.98 [0–31.8]	16.28 [0–53.5]	0.93	
<i>P</i>		0.43	0.57		0.89

PLAC, placebo group; BUD, budesonide group.

*Geometric mean.

†Paired *t*-test for comparison between days –4 and 15 within each group.

‡Unpaired *t*-test for between-group analysis of changes in cell numbers between days –4 and 15 (Δ).

§*P*-value for unpaired *t*-test for between-group analysis at both time points.

Table 3. Cell counts in lamina propria

Marker	Group	Pre-allergen day -4 (mean* [range])	Post-allergen day 15 (mean [range])	Paired <i>t</i> -test† <i>P</i>	Unpaired <i>t</i> -test Δ‡ <i>P</i>
EG2	PLAC	11.93 [1.6–55.3]	17.96 [5.0–54.9]	0.09	
	BUD	10.21 [1.7–36.3]	4.26 [0.6–11.5]	0.002	
P _S	PLAC	0.66	<0.001		<0.001
	BUD	36.77 [8.7–106.1]	34.42 [3.2–89.5]	0.86	
Elastase	PLAC	43.17 [9.8–151.5]	31.48 [14.5–51.2]	0.09	
	BUD	0.54	0.75		
P	PLAC	20.04 [9.1–43.9]	18.81 [4.9–44.2]	0.71	
	BUD	23.77 [12.1–53.6]	17.61 [6.2–65.1]	0.12	
CD68	PLAC	0.35	0.80		0.37
	BUD	21.48 [5.6–42.1]	24.52 [7.5–58.2]	0.54	
P	PLAC	23.87 [5.3–51.6]	19.48 [10.3–51.8]	0.12	
	BUD	0.64	0.28		
CD3	PLAC	93.03 [16.1–190.9]	98.51 [58.9–143.5]	0.73	
	BUD	85.27 [40.7–127.6]	58.93 [32.2–98.8]	<0.001	
P	PLAC	0.68	0.001		0.038
	BUD	96.27 [31.2–183.2]	121.06 [66.6–210.9]	0.22	
CD4	PLAC	107.30 [12.3–219.0]	70.18 [36.5–137.3]	0.07	
	BUD	0.66	0.005		
P	PLAC	30.21 [5.4–66.0]	35.61 [11.2–105.1]	0.52	
	BUD	25.35 [8.7–55.7]	20.93 [0–64.9]	0.56	
CD8	PLAC	0.51	0.15		0.39
	BUD				

PLAC, placebo group; BUD, budesonide group.

*Geometric mean.

†Paired *t*-test for comparison between days -4 and 15 within each group.‡Unpaired *t*-test for between-group analysis of changes in cell numbers between days -4 and 15 (Δ).§*P*-value for unpaired *t*-test for between-group analysis at both time points.

numbers of EG2⁺, CD3⁺, and CD4⁺ cells were significantly different between both groups on day 15 ($P < 0.001$, $P = 0.001$, and 0.005 , respectively). During the study, the changes in the number of EG2⁺, CD3⁺, and CD4⁺ cells between both groups were also significantly different ($P < 0.001$, $P = 0.038$ and 0.03 , respectively). The numbers of elastase⁺, CD8⁺, CD68⁺, and AA1⁺ cells did not change significantly in the lamina propria during the study.

Structural changes

Mean density of proteoglycans and fibronectin in lamina propria (including the reticular basement membrane) The overall staining pattern showed a diffuse matrix staining with a fibrillar pattern in the submucosal area, with variable deposition under the epithelial cell layer (Fig. 2). The epithelial, endothelial, inflammatory, and smooth muscle cells were consistently negative. Baseline values of the mean density of the PG_S biglycan and decorin were significantly different between the asthmatics and the normal control group. The mean density of biglycan was higher in the asthmatics as compared with the normal control group ($P < 0.001$), whereas the mean density of decorin was lower in the asthmatics ($P = 0.005$). There were no significant changes in the mean density of the PG_S after low-dose allergen exposure in the placebo group. However, we did observe an increase in the mean density for both biglycan and versican (0.035 and $P = 0.042$, respectively) in the steroid group. There were no significant changes present in the mean density of

fibronectin, lumican, and decorin in the placebo group as well as the steroid group.

Thickness of the reticular basement membrane The reticular basement membrane was significantly thicker in the asthmatics as compared with the normal control group (mean thickness [μm] ± SEM; asthmatics: 9.39 ± 0.38 vs. normals: 6.07 ± 0.25 , $P = 0.001$). However, there were no significant changes in the thickness of the reticular basement membrane observed after low-dose allergen exposure with or without inhaled steroid-treatment ($P > 0.1$).

Number of goblet cells in the epithelium The asthmatics had a significantly higher number of goblet cells per μm reticular basement membrane (RBM) at baseline as compared with the normal control group (mean number/μm RBM ± SEM; asthmatics: 0.090 ± 0.008 vs. normals: 0.042 ± 0.01 , $P = 0.003$). Although there were no significant changes during low-dose allergen exposure, with or without inhaled steroid treatment, the steroid-group had a significantly lower number of goblet cells in the epithelium as compared with the placebo group at the end of the study (steroid group: 0.055 ± 0.01 vs. placebo-group: 0.089 ± 0.01 , $P = 0.01$).

Discussion

In this study, we demonstrated the effects of asymptomatic repeated low-dose allergen exposure with and without inhaled

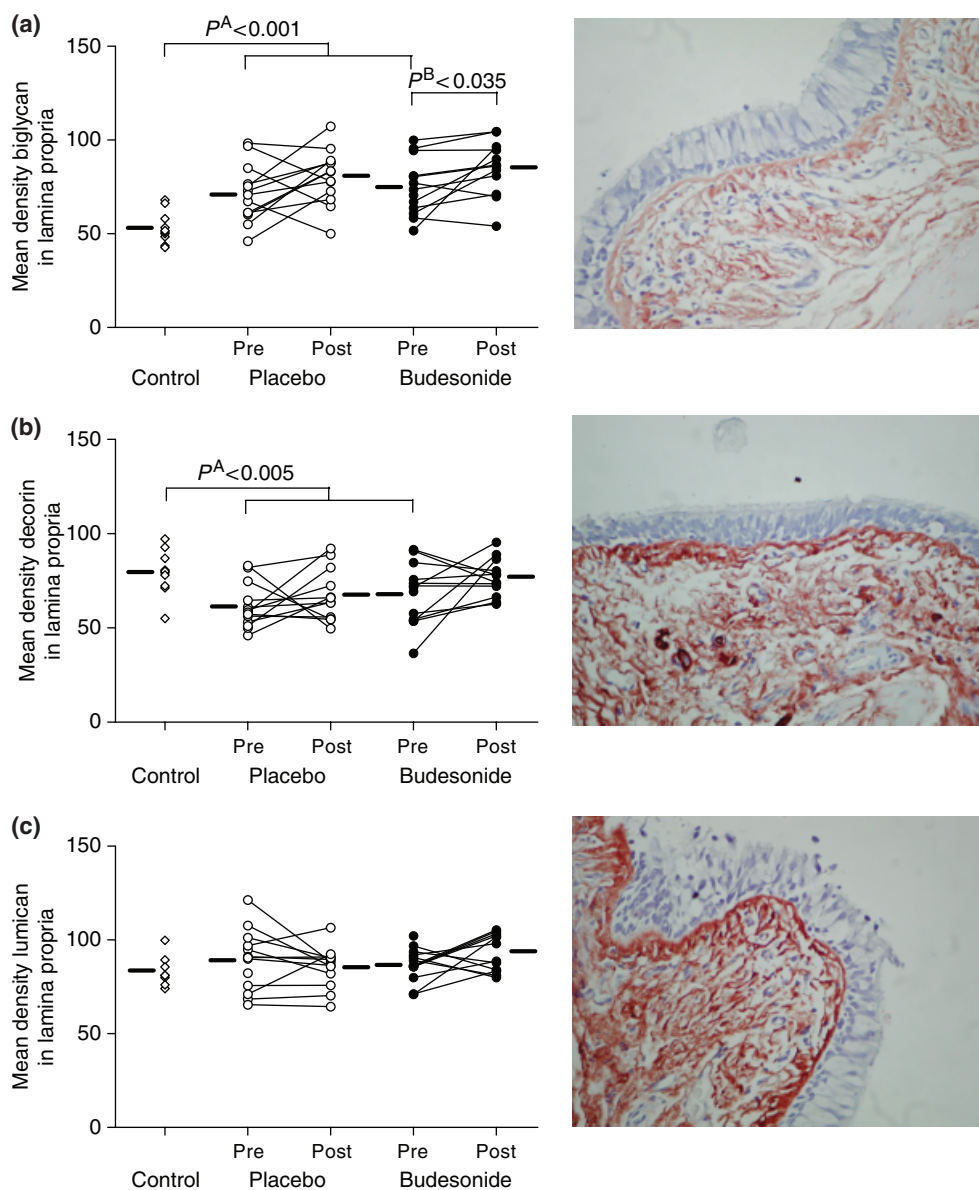


Fig. 2. Individual values of the mean density (0–255 greyvalues) of the PGs biglycan (a), decorin (b), lumican (c), versican (d), and fibronectin (e) in the lamina propria (including the reticular basement membrane) in the normal control group and before (pre: day –4) and after (post: day 15) two weeks of repeated allergen exposure in the placebo-treated and budesonide-treated asthmatic subjects. The mean is depicted as a horizontal bar. P -values are depicted as differences in baseline values between the asthmatics and the normal control group (P^A) within group values (P^B) and changes in density between groups (P^C). Photomicrographs showing examples of stainings in bronchial biopsies of patients with atopic asthma are depicted next to the graph. Original magnification: 400 $\times$.

steroid treatment on bronchial inflammatory and structural changes in mild asthma. Low-dose allergen exposure resulted in a significant increase of epithelial macrophages. Despite this exposure, the numbers of mucosal eosinophils, neutrophils, and T lymphocytes were significantly decreased by inhaled steroid treatment. Interestingly, steroid treatment during allergen exposure increased the mean density of the PGs biglycan and versican. Our findings suggest that daily allergen exposure alone induces bronchial inflammatory changes, although these are limited in mild asthma. The data may implicate that steroids not only prevent worsening of bronchial inflammation but that they also alter airway structure in asthma by increasing the PG_S content.

To our knowledge, we are the first to examine the effects of two weeks of repeated allergen exposure with and without inhaled steroid treatment in bronchial biopsies of patients with asthma. The significant increase of epithelial macrophages after low-dose allergen exposure is in line with previous results of Lensmar et al. [7], who found an altered alveolar macrophage phenotype pattern in bronchoalveolar lavage (BAL) after repeated low-dose allergen exposure in asthmatics. Earlier studies showed that steroid treatment can have anti-inflammatory effects in sputum or BAL despite ongoing allergen exposure in asthmatics [8, 32], and our study extends these findings to airway tissue inflammation. The increased mean density of the PG_S following steroid

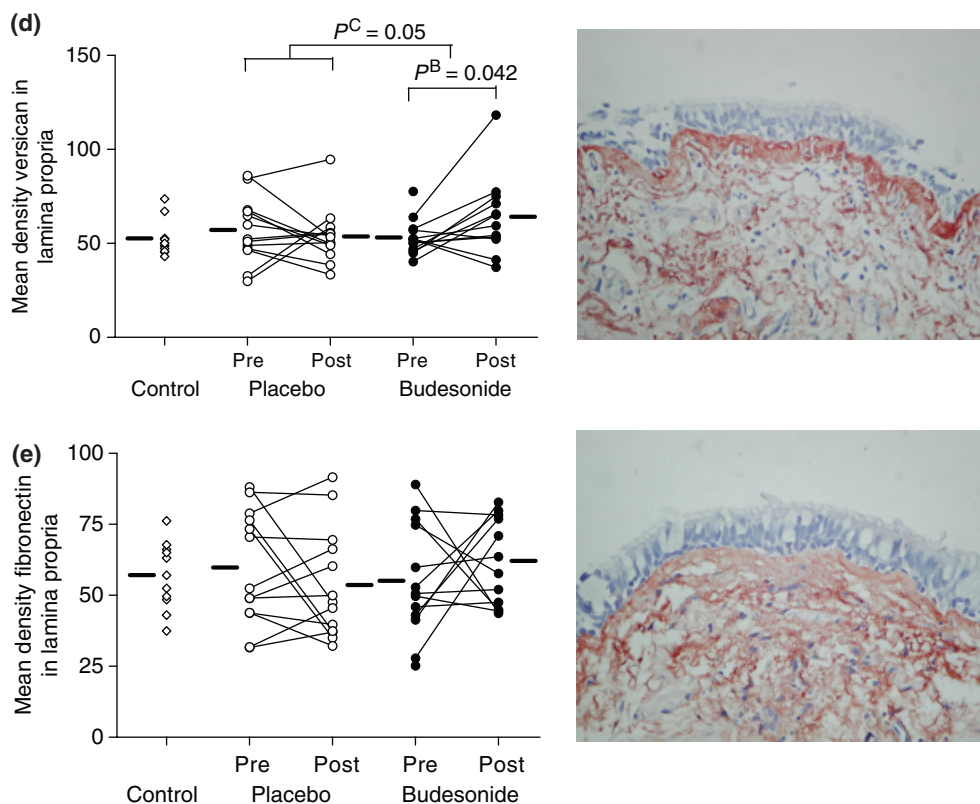


Fig. 2. (Continued).

treatment, during allergen exposure, in our study is also a novel finding, and is in agreement with *in vitro* studies showing a steroid-induced increased synthesis of PGs by chondrocytes [33], and a dose-dependent increase in the production of various ECM proteins, among which are PGs, by human passively sensitized airway smooth-muscle cells [21]. Hence, it may not be surprising that steroid treatment alters ECM proteins, and thereby the airway structure.

We observed relatively minor histological changes after 2 weeks of low-dose allergen exposure. First, the administered allergen dose was very low. This was done on purpose, to mimic chronic allergen exposure without inducing loss of control or an exacerbation of the disease [9]. This could be indicative of the presence of a threshold for developing and/or increasing a mucosal inflammatory cell infiltrate in patients with mild asthma. Second, low-dose allergen exposure during 2 weeks might be relatively short. However, this time period was sufficient in animal models to induce inflammatory changes and changes in fibronectin and PGs [10, 11]. Finally, the fact that we did observe increased numbers of sputum eosinophils in this same group of patients, which was induced 24 h after an allergen dose [9], may indicate that the migration of eosinophils into the lumen had already occurred before the biopsies were taken [34]. We cannot exclude that part of our present results of increased density of the ECM components versican and biglycan may have been influenced by decreased tissue oedema after steroid treatment. However, the fact that no similar significant changes occurred in the mean density of other analysed ECM components after budesonide treatment argues against the fact that oedema alone was responsible for our findings.

Our cellular findings are of interest with regard to a potential role of the macrophage in asthma. It has been shown that macrophage chemotactic peptide (MCP-1) is increased in BAL following endobronchial allergen challenge [35], which could explain the increase in epithelial macrophages in our study. Macrophages may also be involved in airway structural changes through the secretion of metalloproteinases that can degrade various ECM macromolecules including elastin [36], and through the secretion of growth factors such as TGF- β [37].

There are only very few studies with data on the PG content of the ECM in bronchial tissue obtained from asthmatics [19–22]. Previous studies have shown a negative correlation between the expression of the PGs biglycan, lumican and versican in the airways and BHR in asthma [20, 22], whereas this has not been found for decorin [22]. It has recently been demonstrated that fibroblasts from asthmatic patients subjected to mechanical strain enhance PG message [38]. However, it is still unknown whether alterations in the content of the PGs have beneficial or detrimental consequences for structure–function relationships of the airways. What could be the explanation for our present finding, that patients with asthma show a lower decorin and higher biglycan density in the lamina propria as compared with normal controls? First, this could be because of a higher pro-fibrotic TGF- β level in the airways of asthmatics, as it has been shown that TGF- β stimulates synthesis of biglycan while inhibiting synthesis of decorin by lung fibroblasts [39]. Interestingly, in turn, the lower density of decorin in asthma could be a cause of an increased activity/level of TGF- β . Several types of PGS bind to growth factors and modulate

their activities. An anti-fibrotic effect of the SLRP decorin, which can suppress the bioactivity of TGF- β , has been shown [23, 24]. Our results suggest that this may not only be indicative of a role of decorin in TGF- β -mediated fibrotic diseases, including lung fibrosis [16, 24, 40, 41], but also for airway diseases such as asthma.

Although both the SLRPs decorin and biglycan bind TGF- β [23], this may not give the same result [41]. Genetic augmentation of endogenous biglycan expression using adenovirus constructs increased PG and collagen content, whereas this was not shown for decorin [42]. This could indicate that biglycan is able to make the ECM more susceptible to structural changes [20, 22]. Decorin, on the other hand, has even been suggested as having a potential role in the future treatment of fibrotic disorders of the lung [41]. Thus, the decreased mean density of decorin and the increased mean density of biglycan in the airway mucosa may facilitate the structural changes in patients with asthma.

Several studies have shown that steroids may affect biosynthesis of PGs. Depending on the cell type studied, both inhibitory [43, 44] and stimulatory [21, 33] effects have been demonstrated. In our study, the steroid-induced increase in PGs in the ECM of the airways may enhance airway wall thickness and, therefore, promote excessive airway narrowing in asthma [45]. In contrast, versican deposited within and internal to the airway smooth muscle together with the pro-fibrotic effects of biglycan [42] may limit smooth muscle shortening by increasing compressive stiffness of the inner airway wall [46]. In this way, a steroid-induced increase in PGs could be both beneficial and detrimental.

What are the clinical implications of our findings with regard to the long-term effects of inhaled steroids on ECM composition? In severe asthmatics, Chakir et al. [47] could not demonstrate a decrease in collagens I and III content in the bronchial mucosa after 2 weeks of treatment with oral steroids. Interestingly, intervention with anti-IL-5 has been shown to reduce the bronchial subepithelial expression of ECM proteins such as tenascin, lumican, and procollagen III in asthma [48]. Although features of structural changes in the airways of patients with asthma seem to be present already in childhood [49], it is important to elucidate which elements of the remodelling process are progressive later in life, and especially which elements can be prevented. Our results suggest that the current first-line therapy by inhaled steroids in asthma adequately prevents progressive inflammation during long-term allergen exposure in asthma. However, such a therapy is changing ECM protein composition within the airway wall. This may or may not be beneficial for (the decline of) airway function in asthma. Hence, it seems to be mandatory to examine closely the long-term effects of steroid treatment on airway structure and function in this disease.

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