ORIGINAL ARTICLE

Arch. Immunol. Ther. Exp., 2008, **56**, 401–408
PL ISSN 0004-069X

DOI 10.1007/s00005-008-0044-z

Bronchial hyper-responsiveness, subepithelial fibrosis, and transforming growth factor-β_1 expression in patients with long-standing and recently diagnosed asthma

Aneta Tomkowicz[1], Maria Kraus-Filarska[2], Julia Bar[3], Jerzy Rabczyński[4], Michał Jeleń[4], Paweł Piesiak[5], Andrzej Fal[2] and Bernard Panaszek[2]

[1] Department of Dietetics, Faculty of Public Health, Wrocław Medical University, Wrocław, Poland
[2] Department of Internal Medicine and Allergology, Wrocław Medical University, Wrocław, Poland
[3] Department of Clinical Immunology, Wrocław Medical University, Wrocław, Poland
[4] Department of Pathological Anatomy, Wrocław Medical University, Wrocław, Poland
[5] Department of Pulmonology and Lung Cancer, Wrocław Medical University, Wrocław, Poland

Received: 2008.04.29, **Accepted:** 2008.09.30, **Published online first:** 2008.12.01

Abstract

Introduction: Chronic inflammation in asthmatic airways leads to bronchial hyper-responsiveness (BHR) and the development of structural changes. Important features of remodeling include the formation of subepithelial fibrosis due to increased collagen deposition in the reticular basement membrane. Transforming growth factor (TGF)-β might be a central mediator of tissue fibrosis and remodeling.
Materials and Methods: Immunohistochemistry was used to measure collagen III deposition and TGF-β_1 expression in biopsies from patients with long-standing asthma treated with inhaled corticosteroids, patients with recently diagnosed asthma, and control subjects. Computer-assisted image analysis was used to evaluate total basement membrane (TBM) thickness.
Results: Asthmatics, particularly those with long-standing asthma, had thicker TBMs than healthy subjects. Collagen III deposition was comparable in the studied groups. BHR was not correlated with features of mucosal inflammation and was lower in steroid-treated patients with long-standing asthma than in subjects with newly diagnosed asthma untreated with steroids. Epithelial TGF-β_1 expression negatively correlated with collagen III deposition and TBM thickness.
Conclusions: The study showed that TBM thickness, but not collagen III deposition, could be a differentiating marker of asthmatics of different disease duration and treatment. The lack of correlation between BHR and features of mucosal inflammation suggests the complexity of BHR development. Corticosteroids can reduce BHR in asthmatics, but it seems to be less effective in reducing subepithelial fibrosis. The role of epithelial TGF-β_1 needs to be further investigated since the possibility that it plays a protective and anti-inflammatory role in asthmatic airways cannot be excluded.

Key words: bronchial hyper-responsiveness, subepithelial fibrosis, airway remodeling, transforming growth factor-β.

Corresponding author: Aneta Tomkowicz, Ph.D. M.D., Department of Dietetics, Faculty of Public Health, Wrocław Medical University, Bartla 5, 51-618 Wrocław, Poland, e-mail: anewla@wp.pl

INTRODUCTION

Airway inflammation and bronchial hyper-responsiveness (BHR) are the hallmarks of asthma. The inflammatory changes are regarded as a key factor in BHR; however, the relationships between inflammation markers and BHR are not clear, with comparable numbers of reports showing or denying such relationships. Structural changes in the airways are thought to be involved in the development of BHR, particularly in long-standing disease, but the relationship between remodeling features and BHR needs to be further investigated. Impaired functioning of the hypothetical epithelial-mesenchymal trophic unit is thought to play an important role in remodeling pathogenesis [16]. The process of the development of structural changes is thought to be responsible for the irreversible airway obstruction. One of the most prominent histopathological features of remodeling is subepithelial fibrosis due to the deposition of a number of extracellular matrix (ECM) proteins, including collagens

I, III, and V, fibronectin, tenascin, and proteoglycans in the reticular basement membrane (RBM) [29]. Although these features are well recognized, the mechanisms leading to remodeling and the effect of therapy on preventing or reversing these changes are not well understood. A number of different cells and mediators are involved in airway inflammation and remodeling, forming a complex network of multidirectional reactions. It has been postulated that transforming growth factor (TGF)-β is one of the important mediators involved in allergic inflammation and remodeling. It is thought to be involved in the downregulation of activated inflammatory cells, tissue differentiation, and wound healing. In the airways, TGF-β is found in various cell types, including epithelial cells and inflammatory cells beneath the basement membrane. There is speculation about various TGF-β functions at its different locations [21]. There are also contradictory data as to the distribution of TGF-β in the airways of asthmatics. All these discrepancies were reasons to carry out the present study. We investigated BHR, subepithelial fibrosis, features of mucosal inflammation, and TGF-β_1 expression in patients with different asthma durations and different applied treatments.

MATERIALS AND METHODS

Subjects

Thirty-four patients with asthma fulfilling the criteria of the Global Initiative for Asthma of the NHLBI/WHO were recruited (Table 1). They were further divided into two groups: the long-standing asthma group (LSA), with 16 subjects with asthma duration of at least 4 years treated with inhaled corticosteroids (ICS), and the recently diagnosed asthma group (RDA), with 18 subjects with asthma duration of less than 4 years untreated with ICS. The control group consisted of 13 subjects with no history of asthma (Table 1). The study was approved by the Ethics Committee of the Wrocław Medical University and written informed consent was obtained from all subjects before entry into the study.

Lung function and challenge procedure

Pulmonary function tests included three measurements of FEV_1, FEV_1/FVC, and PEF with a flow-screen spirometer (Jaeger GMBH & CoKH, Germany). The reversibility of airway obstruction was investigated in response to inhaled salbutamol. Histamine challenge tests were carried out using computer software with a special protocol developed at the Department of Internal Medicine and Allergology, Wrocław Medical University [20], with a De Vilbiss 646 nebulizer.

Fiberoptic bronchoscopy

Bronchoscopy was performed according to BTS guidelines [7]. The subjects were premedicated with 0.5 mg atropine s.c. and 25 mg pethidine i.v. During bronchoscopy, visual assessment of airway inflammation was

Table 1. Subjects' characteristic

	LSA group n=16	RDA group n=18	Controls n=13
Age (years)*	49.10±15.50	41.6±13.5	46.92±14.7
Sex: F/M	15/1	17/1	10/3
Asthma duration (years)	14.50±12.70	1.7±1.3	–
Asthma severity:			
mild	8 (50%)	16 (88.8%)	–
moderate	5 (31.2%)	2 (11.1%)	–
severe	3 (18.7%)	0	–
Atopy/no atopy	9/7	8/10	2/11
PC20 (mg/ml)	8.89±6.40	4.68±2.9	>16
FEV1 (% pred. value)	85.75±19.40	99.3±17.4	109.79±9.1
PEF (% pred. value)	67.27±18.20	86.5±23.1	88.6±12.4
FEV1%FVC	75.72±13.57	79.34±8.8	82.4±10.0
ICS doses:**			
small: 200–600 μg/die	16.6%	–	–
moderate: 600–1000 μg/die	41.6%	–	–
large: ≥1000 μg/die	41.6%	–	–
ICS use length (years):			
range/mean	1–12.5/(4.9±3.58)	–	–
Smoking cigarettes history			
positive (%) (always <10 pack-years)	18.7%	22.2%	0%

ICS – inhaled corticosteroids, LSA – long–standing asthmatics treated with ICS, RDA – recently diagnosed asthmatics untreated with ICS.

* Values are expressed as means ±SEMs, except for cases where showed differently, **ICS dose during last 3 months (budesonide).

done and the macroscopic bronchitis index was derived (0–3 scale) depending on the presence of abnormal mucosal features [28]. Two to three biopsies were collected from the subcarinae of the right upper lobe using a Pentax FB18X bronchoscope, fixed in formalin solution, and embedded in paraffin.

Tissue processing and immunohistochemistry

Biopsy specimens coded prior to analysis were studied histologically and immunohistochemically. A histopathologist assessed the features of mucosal inflammation and on that basis the microscopic inflammation index was introduced. This index was used to differentiate the degree of mucosal inflammation in the biopsies and to compare it with the macroscopic inflammation index (bronchitis index according to Thompson et al. [28]). The microscopic inflammation index was evaluated semiquantitatively by the histopathologist on the basis of the presence of inflammatory cells in the mucosa (1 – small inflammation, 2 – moderate, 3 – large degree of inflammation).

For immunohistochemistry, the sections were stained using the streptavidin-biotin-peroxidase technique [26]. To assess TGF-β_1 and collagen type III expression the sections were incubated with a mouse anti-TGF-β_1 monoclonal antibody (dilution 1:20, Novo Castra Laboratories, England) and with a mouse anti-collagen III monoclonal antibody (dilution 1:100, Medicorp, Canada), respectively. All the sections were then incubated with biotinylated antibody (LSAB Kit, Dako, USA) and with a streptavidin-biotin-peroxidase complex (LSAB Kit, Dako, USA). They were subsequently developed in 3,3-diaminobenzidine (DAB, Liquid K, Dako, US). Skin samples were used as a positive control for anti-collagen III antibodies and the placenta for anti-TGF-β_1 antibodies. Sections in which the primary antibodies were omitted served as a negative control.

Immunohistochemical staining was assessed with an Olympus BH-2 light microscope at a magnification of 200× in a semiquantitative analysis with the visual scale. Two parameters were used to determine TGF-β_1 expression and collagen type III deposition within the epithelium and subepithelial area in the airways. The first was the extent of the tissue area which was stained immunohistochemically and is presented as the percentage of the total tissue area and the second was the intensity of the immunohistochemical staining. Both were assessed semiquantitatively. The staining intensity was assessed on a scale of 0 to 3 (0 – no expression, 1 – poor expression, 2 – moderate expression, 3 – strong expression). Both these parameters correlated positively with each other.

Total basement membrane (TBM) thickness (the "true" basement membrane and RBM together) was assessed on sections stained with toluidine blue using a Multiscan 08.98 computer-assisted image analysis system, making 10 measurements for each patient along the basement membrane from the base of the epithelium to the outer limit of the RBM.

Statistical analysis

MS EXCEL software was used for the statistical analysis. Results are given as the mean ±SD. Of the analyzed features, BHR, TBM, and collagen III deposition had normal distributions, while epithelial and subepithelial TGF-β_1 expressions were right-skewed, so a logarithmic transformation was used. Student's *t*-test was applied to compare two groups and the chi-squared test to assess the frequency in the groups. Additionally, Fisher's exact test as well as Scheffe's and Levene's tests were performed.

RESULTS

The groups did not differ with regard to age or sex. Lung function parameters were significantly lower in the LSA group than in the RDA group (FEV_1%: $p=0.039$, FVC%: $p=0.012$). Patients with asthma showed a negative correlation between FEV_1 and asthma duration ($p=0.004$; Fig. 1).

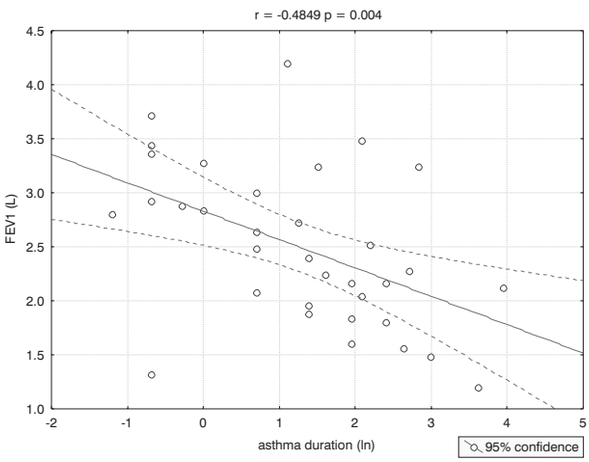

Fig. 1. Scatterplot: astma duration (ln) vs. FEV1.

The mean PC20 value in all asthmatics was 6.58±5.24 mg/ml. Mean PC20 was significantly lower in the RDA group than in the LSA group ($p=0.02$; Table 1). A significant positive correlation between PC20 values and ICS treatment duration was observed ($p<0.05$; Fig. 2). No correlations were found between PC20 values and spirometric indices, asthma duration, TGF-β_1 expression, TBM thickness, or collagen type III mucosal expression. PC20 values were not significantly differentiated with regard to microscopic or macroscopic features of mucosal inflammation.

The patients with asthma had thicker TBM (mean: 8.56±3.11 μm) than the control subjects (3.25±0.96 μm; $p<0.001$; Table 2). Moreover, in spite of steroid treatment, there was significantly thicker TBM in the LSA group than in the RDA group (Table 2). Significant pos-

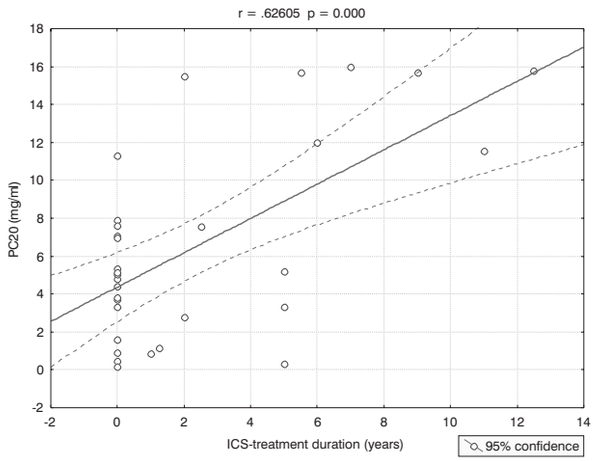

Fig. 2. Scatterplot: ICS-treatment duration vs. PC20.

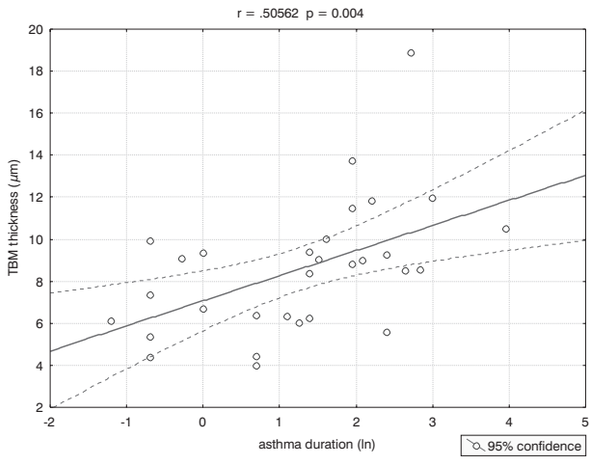

Fig. 3. Scatterplot: astma duration vs. TBM thickness.

Table 2. Total basement membrane thickness and collagen III deposition measurements

	TBM (μM)	Subepithelial collagen III	
		expression (%)	intensity
LSA (ICS+)	10.52±3.11	59.0±42.6	1.70±1.13
RDA	6.85±1.89	65.29±34.2	1.76±0.93
Controls	3.25±0.96	54.16±39.8	1.66±0.88
p values	<0.001*	0.74	0.8

All values are expressed as means ±SD. LSA (ICS+) – long-standing asthma group treated with ICS, RDA – recently diagnosed asthma, TBM – total basement membrane.
* Statistically significant.

itive correlation was found between TBM thickness and asthma duration (p=0.004; Fig. 3). There was no clear correlation between TBM thickness and lung function (FEV_1).

The expression of collagen type III in the subepithelial area was similar in the studied groups (Table 2). No correlations were found between collagen III deposition and lung function. There were also no significant correlations observed between TBM thickness and collagen III deposition.

TGF-β_1 was rarely expressed in the epithelial cells of asthmatic airways, particularly in the LSA group compared with control subjects (Table 3), although these differences were not statistically significant. In the LSA group, negative correlation was found between TGF-β_1 epithelial expression and collagen III deposition (p=0.03; Fig. 4) and TBM thickness (p=0.05; Fig. 5). In the RDA group, positive correlation between epithelial TGF-β_1 expression and lung function was observed (Fig. 6).

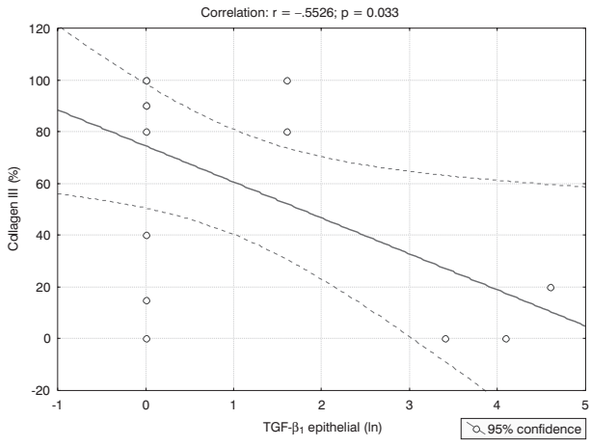

Fig. 4. Scatterplot: TGF-β_1 epithelial vs. collagen III in LSA group.

Examples of bronchial mucosal biopsies stained with toluidine blue and antibodies to collagen type III are shown in Figs. 7–11.

Table 3. TGF-β_1 expression in the airways

	All asthmatics	LSA (ICS+)	RDA	Controls
Number	34	16	18	11
TGF-β_1 epithelial expression (%)	10.58 ± 24.2	9.06 ± 24.09	11.94 ± 24.91	25.45 ± 36.70
TGF-β_1 subepithelial expression (%)	12.20 ± 24.03	6.56 ± 10.11	17.22 ± 31.21	16.80 ± 27.40
TGF-β_1 mucosal intensity*	0.83 ± 0.89	0.81 ± 0.91	0.86 ± 0.90	0.81 ± 0.91

All values are expressed as means ±SD; LSA(ICS+) – long –standing asthma group, RDA – recently diagnosed asthma.
Mucosal intensity – analysis of epithelial and subepithelial area together.
All differents not statistically significant (p>0.05).

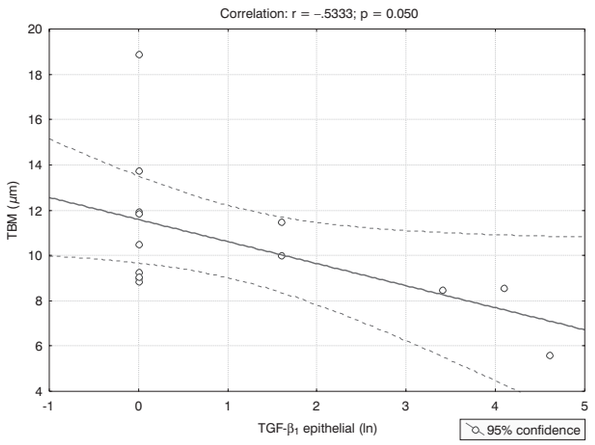

Fig. 5. Scatterplot: TGF-β_1 epithelial vs. TBM in LSA group.

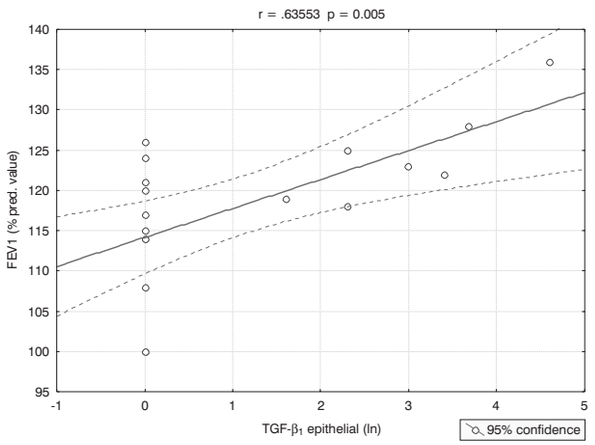

Fig. 6. Scatterplot: TGF-β_1 epithelial vs. FEV1 in the RDA group.

DISCUSSION

This study shows that in patients with long-standing asthma, BHR, probably as a result of corticosteroid treatment, is significantly decreased compared with steroid-naive patients with recently diagnosed disease. This is consistent with a number of previous studies [12, 27, 31]. Moreover, significant correlation between steroid therapy duration and PC20 values was found.

In the present study we did not find any differences in PC20 values with regard to microscopic or macroscopic features of mucosal inflammation. Our investigation was semiquantitative as we did not count the numbers of inflammatory cells in the mucosa, but it is in keeping with previous studies. The results of other studies on BHR and airway inflammation are largely inconsistent, with equal numbers of positive [10] and negative reports [19]. Our results are consistent with current beliefs that BHR development depends not only on airway inflammation, but also on airway smooth muscle disorders or other unknown agents [4, 14]. Bousquet et

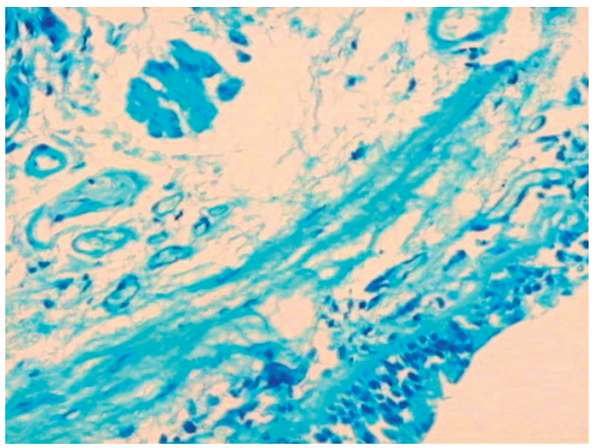

Fig. 7. Photomicrograph of a bronchial biopsy from a patient with long-standing atopic asthma (staining with toluidine blue) showing thickening of the basement membrane and deposition of extracellular matrix below the basement membrane.

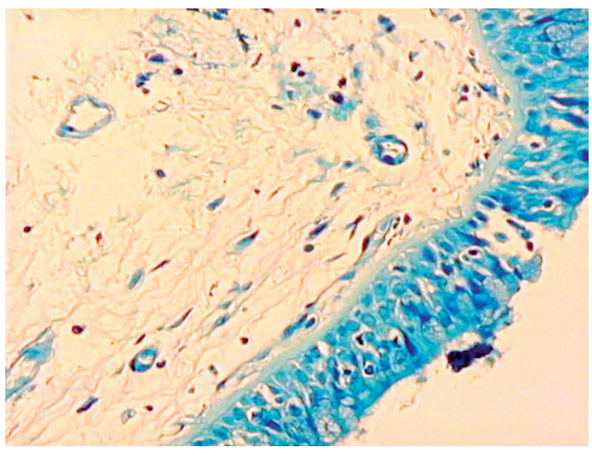

Fig. 8. Photomicrograph of a bronchial biopsy from a healthy subject (staining with toluidine blue) showing epithelium, basement membrane, and a small amount of the subepithelial extracellular matrix.

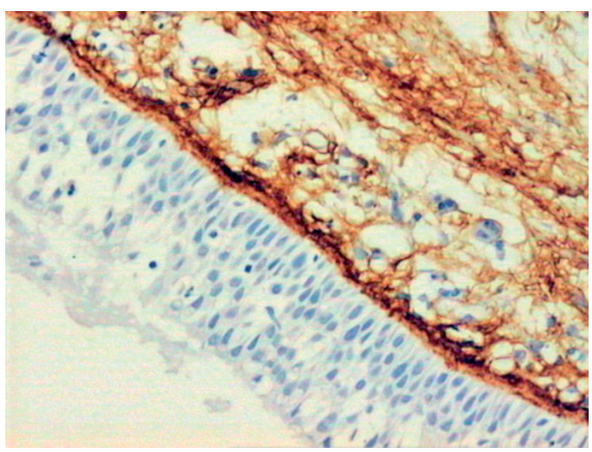

Fig. 9. Photomicrograph of a bronchial biopsy from a patient with long-standing atopic asthma showing immunohistochemical staining with antibodies to collagen type III (brown collagen III fibers).

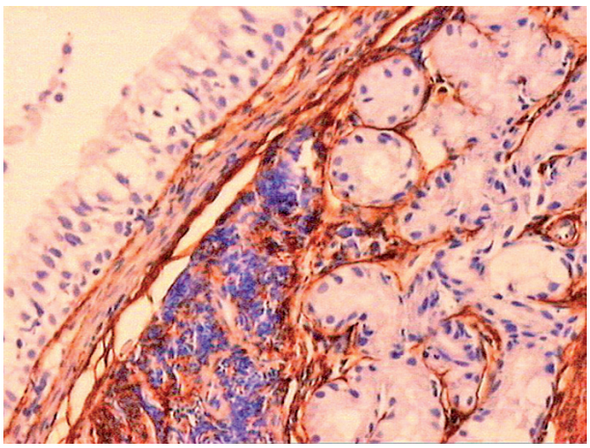

Fig. 10. Photomicrograph of a bronchial biopsy from a patient with recently diagnosed asthma showing immunohistochemical staining with antibodies to collagen type III (brown collagen III fibers).

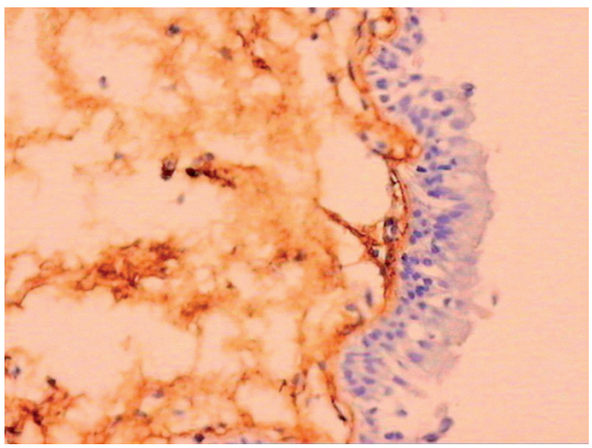

Fig. 11. Photomicrographs of a bronchial biopsy from a healthy subjectc showing immunohistochemical staining with antibodies to collagen type III (brown collagen III fibers).

al. [6] proposed the hypothesis that in asthmatics, BHR reflects a complex relationship between bronchial inflammation and remodeling, which makes it difficult to demonstrate a simple correlation between these processes and clinical outcome of the disease. In our study we observed a lack of correlation between the degree of BHR and subepithelial collagen III deposition. This is in keeping with previous studies [5].

It is proven that the "true" basement membrane is of normal structure and thickness in asthmatics. On the other hand, studies comparing asthmatics with normal subjects consistently find thickening of the RBM in asthmatics [2, 9, 11, 15, 17]. There is a wide range of reported values for asthmatics (5.8–30 μm) and control subjects (3.2–7.5 μm). This is probably a matter of the different techniques used.

The role of RBM thickening in BHR development is not clear and the data are conflicting: thickened RBM might be responsible for excessive BHR and airway obstruction, but there are opinions that increased stiffness of the inner wall of the airway due to a thickened RBM might be a protective mechanism against excessive airway narrowing [18, 30].

In the present study we demonstrated thickening of the TBM in patients with asthma, particularly in patients in the LSA group. Significant positive correlation in all asthmatics was found between TBM thickness and asthma duration; however, the corticosteroid therapy applied in the LSA group makes an interpretation of these findings difficult. There are disparities in the previous reports regarding the effects of steroids on RBM thickness [17, 23, 25]. In the present study the effect of corticosteroids on airway remodeling was not directly investigated. We could only indirectly investigate the influence of therapy on clinical and immunohistochemical parameters by observing the steroid-treated group (LSA). Our findings might suggest that corticosteroids could be insufficiently effective in reducing subepithelial fibrosis, which is consistent with previous studies [3, 13, 23], but we cannot exclude the possibility that it could be the result of late treatment introduction.

In the present study we did not find any correlation between TBM thickness and lung function parameters in asthmatics; however, it was seen in all patients and control subjects combined in one group. It is obvious that for this reason the interpretation of the finding is difficult and it could imply that RBM thickening might not be such an important agent affecting airway obstruction. The lack of association between TBM thickness and BHR demonstrated here is in keeping with previous studies [5], but contradictory to others [9, 10, 17]. Our findings could suggest that both these processes might be independent results of airway inflammation, although one cannot exclude the importance of RBM thickening in BHR development.

With regard to subepithelial fibrosis assessment, our findings derived from the collagen III deposition are quite different from those obtained by the assessment of TBM thickness. Collagen III is one of the main components of the RBM in patients with asthma. However, we found no correlation between TBM thickness and collagen III deposition in airway mucosa. The different extents of these two tissue regions could be an explanation, as we investigated collagen III deposition within the RBM and also in the deeper submucosa. Other investigators demonstrated a lack of such association [11]. In the present study we did not find any significant difference in collagen III deposition between the studied groups. Other authors investigated deeper collagen III deposition and obtained similar results [31]. We did not observe any correlation between collagen III deposition and clinical outcome. Considering all the limitations of our study (e.g. small population), our results showing a lack of significant correlation between TBM thickness or collagen III deposition and lung function or BHR could imply that these remodeling components may not be crucial for asthma pathogenesis and clinical outcome.

TGF-β is a profibrotic cytokine which reveals the prevailing anti-inflammatory activity. There is a hypoth-

esis of TGF-β signaling disorders within asthmatic airways which could result in airway inflammation maintenance [1]. The expression of TGF-β in asthmatics varies among investigators [8, 17, 23, 30]. Magnan et al. [22] reported decreased TGF-β expression in the asthmatic epithelium, while others (e.g. Vignola) observed elevated TGF-β expression in the epithelium of asthmatics [30]. In our study we did not find any significant difference in epithelial and submucosal TGF-β_1 expression between the two groups of asthmatics and control subjects. The results of the study confirm those of other investigators [11, 17]. However, it is worth mentioning that we showed an insignificant tendency towards higher epithelial TGF-β_1 expression in healthy subjects compared with asthmatics. There is some evidence that the epithelial TGF-β distribution might be the main normal source of that growth factor in the airways, where it could play an anti-inflammatory role in maintaining the tissue homeostasis [8, 21]. In the present study the protective role of TGF-β_1 might be confirmed by the positive correlation between TGF-β_1 epithelial immunoreactivity and lung function and the negative correlation between that TGF-β_1 distribution and collagen III deposition. These findings suggest that TGF-β_1 in the epithelium might exert a different role from the one it exerts in the ECM, as these correlations were not seen regarding submucosal TGF-β_1 immunoreactivity. In the present study the other agent making the interpretation difficult could be the corticosteroid therapy introduced in LSA-group, as the influence of ICS on TGF-β_1 expression is not clear [3, 21, 24].

Taking into account all the limitations of our study (e.g. partially semiquantitative methodology), we cannot exclude that airway inflammation and BHR, two major features of asthma, might be loosely related to each other. Obviously, performing cell counts would be necessary to confirm this. Subepithelial fibrosis remains a fact in asthmatic airways, but it role and its linkage with clinical parameters are still unclear and further studies are necessary to explain them as well as the influence of corticosteroid treatment on remodeling components. RBM thickening in patients with asthma is just one of a number of remodeling components, and simple relationships between this parameter and clinical outcome of the disease are difficult to find; however, it is a marker differentiating asthmatics and healthy subjects. TGF-β_1 acts in a complex multidirectional net of mediators. Homeostasis within the net and the cooperation of cytokines or growth factors influences its final result. As we suggest, it can have different activity depending on the distribution and the microenvironment.

Acknowledgment: An abstract have been published in Allergy 2007, vol. 62 suppl. 83; p. 140 pos. 369.

REFERENCES

1. Barbato A., Turato G., Baraldo S., Bazzan E., Calabrese F., Tura M., Zuin R., Beghe B., Maestrelli P., Fabbri L. M. and Saetta M. (2003): Airway inflammation in childhood asthma. Am. J. Respir. Crit. Care Med., **168**, 798–803.
2. Benayoun L., Druilhe A., Dombret M. C., Aubier M. and Pretolani M. (2003): Airway structural alterations selectively associated with severe asthma. Am. J. Respir. Crit. Care Med., **167**, 1360–1368.
3. Bergeron C. and Boulet L. P. (2006): Structural changes in airway diseases. Chest, **129**, 1068–1087.
4. Berger P., Girodet P. O. and Manuel Tunon-de-Lara J. (2005): Mast-cell myositis: a new feature of allergic asthma? Allergy, **60**, 1238–1240.
5. Boulet L. P., Turcotte H., Laviolette M., Naud F., Bernier M. C., Martel S. and Chakir J. (2000): Airway hyperresponsiveness, inflammation and subepithelial collagen deposition in recently diagnosed versus long-standing mild asthma. Am. J. Respir. Crit. Care Med., **162**, 1308–1313.
6. Bousquet J., Jeffery P. K., Busse W. W., Johnsson M. and Vignola A. M. (2000): Asthma: From bronchoconstriction to airways inflammation and remodeling. Am. J. Respir. Crit. Care Med., **161**, 1720–1745.
7. British Thoracic Society Bronchoscopy Guidelines Committee, a Subcommittee of Standards of Care Committee of British Thoracic Society (2001): British Thoracic Society guidelines on diagnostic flexible bronchoscopy. Thorax, **56** (suppl. 1), i1–i21.
8. Chakir J., Shannon J., Molet S., Fukakusa M., Elias J., Laviolette M., Boulet L. P., Hamid Q. (2003): Airway remodeling-associated mediators in moderate to severe asthma: effects of steroids on TGF-β, Il-11, Il-17 and type I and type III collagen expression. J. Allergy Clin. Immunol., **111**, 1293–1298.
9. Chetta A., Foresi A., Del Donno M., Bertorelli G., Pesci A. and Olivieri D. (1997): Airways remodeling is a distinctive feature of asthma and is related to severity of disease. Chest, **111**, 852–857.
10. Chetta A., Foresi A., Del Donno M., Consigli G. F., Bertorelli G., Pesci A., Barbee R. A. and Olivieri D. (1996): Bronchial responsiveness to distilled water and methacholine and its relationship to inflammation and remodeling of the airways in asthma. Am. J. Respir. Crit. Care Med., **153**, 910–917.
11. Chu H. W. C., Halliday J. L., Martin R. J., Leung D. Y. M., Szefler S. J. and Wenzel S. E. (1998): Collagen deposition in large airways may not differentiate severe asthma from milder forms of the disease. Am. J. Respir. Crit. Care Med., **158**, 1936–1944.
12. Currie G. P., Fowler S. J. and Lipworth B. J. (2003): A dose response of inhaled corticosteroids on bronchial hyperresponsiveness a meta-analysis. Ann. Allergy Asthma Immunol., **90**, 194–198.
13. De Blic J., Tillie-Leblond I., Tonnel A. B., Jaubert F. and Scheinmann P. (2004): Difficult asthma in children: an analysis of airway inflammation. J. Allergy Clin. Immunol., **113**, 94–100.
14. Dulin N. O., Fernandes D. J., Dowell M., Bellam S., McConville J., Lakser O., Mitchell R., Camoretti-Mercadp B., Kogut P. and Solway J. (2003): What evidence implicates airway smooth muscle in the cause of BHR? Clin. Rev. Allergy Immunol., **24**, 73–84.
15. Fedorov I. A., Wilson S. J., Davies D. E. and Holgate S. T. (2005): Epithelial stress and structural remodelling in childhood asthma. Thorax, **60**, 389–394.
16. Holgate S. T., Holloway J., Wilson S., Buccieri F., Puddicombe S. and Davies D. (2004): Epithelial-mes-

enchymal communication in the pathogenesis of chronic asthma. Proc. Am. Thorac. Soc., **1**, 93–98.
17. Hoshino M., Nakamura Y. and Sim J. J. (1998): Expression of growth factors and remodelling of the airway wall in bronchial asthma. Thorax, **53**, 21–27.
18. Jeffery P. K., Godfrey R. W., Adelroth E., Nelson F., Rogers A. and Johansson S. A. (1992): Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma. A quantitative light and electron microscopic study. Am. Rev. Respir. Dis., **145**, 890–899.
19. Liakakos P., Snell G. I., Ward C., Johns D. P., Bamford T. L., Williams T. J. and Walters E. H. (1997): Bronchial hyperresponsiveness in lung transplant recipients: lack of correlation with airway inflammation. Thorax, **52**, 551–556.
20. Liebhart J., Gladysz U., Krusińska E., Malolepszy J., Liebhart E. and Woźniak M. (1995): The computer assisted histamine bronchoprovocation test. Int. Rev. Allergol. Clin. Immunol., **1**, 30–38.
21. Magnan A., Mege J. L., Escallier J. C., Brisse J., Capo C., Reynaud M., Thomas P., Meric B., Garbe L., Badier M., Viard L., Bongrand P., Giudicelli R., Metras D., Fuentes P., Vervloet D. and Noirclerc M. (1996): Balance between alveolar macrophage Il-6 and TGF-β in lung transplant recipients. Am. J. Respir. Crit. Care Med., **153**, 1431–1436.
22. Magnan A., Retornaz F., Tsicopoulos A., Brisse J., Van Pee D., Gosset P., Chamlian A., Tonnel A. B. and Vervioet D. (1997): Altered compartmentalization of transforming growth factor-beta in asthmatic airways. Clin. Exp. Allergy, **27**, 389–395.
23. Mauroy B., Filoche M., Weibel E. R. and Sapoval B. (2004): An optimal bronchial tree may be dangerous. Nature, **427**, 633–636.
24. McMillan S. J., Xanthou G. and Lloyd C. M. (2005): Therapeutic administration of budesonide ameliorates allergen-induced airway remodelling. Clin. Exp. Allergy, **35**, 388–396.
25. Niimi A., Matsumoto H., Takemura M., Ueda T., Chin K. and Mishima M. (2003): Relationship of airway wall thickness to airway sensitivity and airway reactivity in asthma. Am. J. Respir. Crit. Care Med., **168**, 983–988.
26. Roche W. R., Beasley R., Williams J. H. and Holgate S. T. (1989): Subepithelial fibrosis in the bronchi of asthmatics. Lancet, **1**, 520–524.
27. Shiba K., Kasahara K., Nakajiama H. and Adachi M. (2002): Structural changes of the airway wall impair respiratory functions, even in mild asthma. Chest, **122**, 1622–1626.
28. Thompson A., Huerta G., Robbins S., Sisson J. H., Spurzem J. R., von Essen S., Rickard K., Romberger D. J., Rubinstein I. et al. (1993): The bronchitis index: a semiquantitative visual scale for the assessment of airway inflammation. Chest, **103**, 1482–1488.
29. Tomkowicz A. and Kraus-Filarska M. (2006): Airway remodeling in asthma. Pol. Merkur. Lekarski, **20**, 99–103.
30. Vignola A. M., Chanez P., Chiappara G., Merendino A., Pace E., Rizzo A., La Rocca A. M., Bellia V., Bonsignore G. and Bousquet J. (1997): Transforming growth factor-β expression in mucosal biopsies in asthma and chronic bronchitis. Am. J. Respir. Crit. Care Med., **156**, 591–599.
31. Ward C., Reid D., Orsida B. E., Feltis B., Ryan V. A., Johns D. and Walters E. H. (2005): Inter-relationships between airway inflammation, reticular basement membrane thickening and bronchial hyper-reactivity to methacholine in asthma; a systematic bronchoalveolar lavage and airway biopsy analysis. Clin. Exp. Allergy, **35**, 1565–1571.