

Airway remodeling-associated mediators in moderate to severe asthma: Effect of steroids on TGF- β , IL-11, IL-17, and type I and type III collagen expression

Jamila Chakir, PhD,^a Joanne Shannon, MD,^b Sophie Molet, PhD,^b Motonori Fukakusa, MD,^b Jack Elias, MD,^c Michel Laviolette, MD,^a Louis-Philippe Boulet, MD,^a and Qutayba Hamid, MD, PhD^b *Montreal and Laval, Quebec, Canada, and New Haven, Conn*

Background: Important features of airway remodeling in asthma include the formation of subepithelial fibrosis and increased deposition of types I and III collagen. TGF- β , IL-11, and IL-17 are profibrotic cytokines involved in the formation of subepithelial fibrosis and are increased in patients with asthma, particularly in those with severe disease.

Objective: The purpose of this study was to investigate the effect of corticosteroids on the expression of these profibrotic cytokines and on extracellular matrix deposition.

Methods: We used immunocytochemistry to measure the expression of TGF- β , IL-11, IL-17, and collagen types I and III in the airways of patients with mild asthma (n = 9), patients with moderate-to-severe asthma (n = 10), and control subjects without asthma (n = 6). Baseline bronchial biopsy specimens were obtained in all groups. In addition, repeat biopsies were obtained in the patients with moderate-to-severe asthma after a 2-week course of oral corticosteroids.

Results: TGF- β expression was significantly higher in all groups with asthma, and it did not decrease after treatment with oral corticosteroids. Levels of IL-11 and IL-17 were increased in patients with moderate-to-severe asthma compared with patients with mild asthma and normal controls ($P < .05$). The expression of these cytokines decreased with oral corticosteroids in the moderate-to-severe group to levels that were comparable to those seen in the patients with mild asthma and in the normal controls ($P < .005$). Expression of types I and III collagens was higher in the patients with moderate-to-severe asthma than in the patients with mild asthma and the controls ($P < .05$; $P < .001$). Treatment with corticosteroids did not decrease the expression of types I and III collagens.

Conclusions: These results confirm the association of increased levels of TGF- β , IL-11, IL-17, and types I and III collagens with severe disease and suggest that the failure of corticosteroids to decrease collagen deposition might be due to per-

sistently elevated TGF- β expression. (*J Allergy Clin Immunol* 2003;111:1293-8.)

Key words: Severe asthma, cytokines, epithelial cell, collagen deposition, airway remodeling, corticosteroids

Asthma has a wide clinical spectrum, ranging from mild disease to severe, intractable disease. In its severe form, asthma is characterized by significant loss of lung function and frequent dependence on oral corticosteroids. The airway obstruction observed in asthma is thought to be secondary to airway remodeling. The histopathologic features of this process include epithelial changes, subepithelial fibrosis (thickening of the basement membrane), inflammatory cell infiltration, hyperplasia, and hypertrophy of the bronchial smooth muscle and mucus hypersecretion. One of the most prominent features of airway remodeling is subepithelial fibrosis, and this characteristic is reportedly a potential marker for disease severity.^{1,2} Important mediators involved in the process of airway remodeling are TGF- β , IL-11, and IL-17. TGF- β is a profibrotic cytokine, and the TGF- β isoforms are implicated in the extracellular matrix (ECM) changes observed in fibrosis. In vitro, TGF- β has been shown to stimulate fibroblasts to secrete a number of ECM proteins, including collagen types I and III, fibronectin, tenascin, and proteoglycans.³⁻⁹ Basal levels of TGF- β are increased in bronchoalveolar lavage fluids of patients with asthma, and these levels are further increased in response to allergen exposure.¹⁰ In addition, expression of TGF- β is increased in the airways of patients with asthma and seems to correlate with disease severity and degree of subepithelial fibrosis.¹¹

IL-11 is produced by many cell types, including human lung fibroblasts and alveolar and airway epithelial cells.^{12,13} Tang et al¹⁴ demonstrated that overexpression of IL-11 in the lungs results in subepithelial fibrosis and promotes the accumulation of myocytes and myofibroblasts. More recently, IL-11 expression was found to be increased in the airways of patients with asthma compared with normal controls and seems to correlate with disease severity and a reduced FEV₁.¹⁵ IL-17 is a newly described cytokine and is present in higher levels in the sputum, bronchoalveolar lavage fluid, and eosinophils of patients with asthma compared with controls without asthma. In vitro, IL-17 stimulates human bronchial

From ^aCentre de Recherche, Hôpital Laval, Institut Universitaire de Cardiologie et de Pneumologie, Laval; ^bMeakins-Christie Laboratories, McGill University, Montreal; and ^cYale University, New Haven.

Supported by grants from Le Réseau du FRSQ en Santé Respiratoire and Astra-Zeneca Canada.

Drs J. Chakir and Q. Hamid are recipients of "Chercheur-Boursier" awards from the "Fonds de la Recherche en Santé du Québec."

Received for publication December 4, 2002; revised March 16, 2003; accepted for publication March 26, 2003.

Reprint requests: Qutayba Hamid, MD, PhD, Meakins-Christie Laboratories, McGill University, 3626 St Urbain, Montreal, PQ H2X 2P2, Canada.

© 2003 Mosby, Inc. All rights reserved.

0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.1557

TABLE I. Subjects' characteristics

	n	Age (y)	Sex (F/M)	FEV ₁ (% predicted)	PC ₂₀ (mg/mL)	Atopy (yes/no)
Normal controls	6	26 ± 1	2/4	110 ± 5	152 ± 35	1/5
Patients with mild asthma	9	27 ± 2	0/9	92 ± 3.1	2 ± 21	8/1
Patients with moderate-to-severe asthma	10	31 ± 3	4/6	58.5 ± 3	—	10/0

All values are expressed as means ± SEMs.

TABLE II. Clinical and physiologic responses to oral corticosteroid treatment in patients with moderate-to-severe asthma

	Values
Before oral corticosteroid treatment	
FEV ₁ pre-BD (% prednisolone)	58.5 ± 3
FEV ₁ post-BD (% prednisolone)*	94 ± 1
Percent change vs pre-BD	64.7 ± 12
Serum cortisol (nmol/L)	512 ± 61
On oral corticosteroids†	
FEV ₁ pre-BD (% prednisolone)	73.1 ± 6
% change vs precorticosteroid	25.04 ± 2
FEV ₁ post-BD (% prednisolone)*	91.3 ± 4
% change vs pre-BD	29.7 ± 4
Cortisol (nmol/L)	24.2 ± 4

All values are expressed as means ± SEMs.

*BD, inhaled salbutamol 200 µg.

†Two weeks on 40 mg/day methylprednisolone.

Abbreviation used

ECM: Extracellular matrix

fibroblasts to synthesize and release higher amounts of IL-6 and IL-11, which are 2 profibrotic cytokines.¹⁶

Although a number of reports describe the effects of steroids on subepithelial fibrosis, the impact of corticosteroids on the expression of profibrotic cytokines in severe asthma has not yet been explained.¹⁷ The objective of this study was to investigate the expression of TGF-β, IL-11, IL-17, and types I and III collagens in patients with asthma with varying disease severity. In addition, we studied the effect of oral corticosteroids on the expression of these profibrotic cytokines. Our data provide additional evidence that a correlation exists between disease severity in asthma and the expression of TGF-β, IL-11, IL-17, and types I and III collagens. Furthermore, we show for the first time that IL-17 levels also correlate with disease severity. In addition, our data demonstrate that treatment with oral corticosteroids significantly reduces the expression of IL-11 and IL-17 in the airways of patients with moderate-to-severe asthma. In contrast, this treatment did not affect type I and type III collagen deposition or the expression of TGF-β. This suggests that the failure of oral corticosteroids to decrease collagen deposition might be related, in part, to the ongoing expression of TGF-β.

METHODS

Subjects

Nineteen patients with asthma, as defined by the American Thoracic Society criteria,¹⁸ were recruited from the Asthma Clinic at the Laval Hospital (Laval, Quebec, Canada) (Table I). Atopy was defined as a positive skin reaction to at least 1 common allergen. Patients in the mild asthma group (n = 9) had a baseline FEV₁ of >75% and were treated with a short-acting β₂-agonist as needed and less than 3 doses a week. The patients in the moderate-to-severe asthma group (n = 10) had a baseline FEV₁ of <80% predicted with a >30% bronchodilator response (Table II). None of these subjects had been treated with oral prednisone during the 6 months preceding the study. All subjects were treated with inhaled β₂-agonists and were not exposed to inhaled steroids or leukotriene receptor antagonist during the month preceding the study. Patients in the moderate-to-severe group were treated with a 2-week course of oral corticosteroids (methylprednisolone 40 mg/day). Compliance with oral corticosteroid therapy was confirmed with plasma morning cortisol levels. The control group (n = 6) consisted of healthy volunteer subjects with no history of allergies or asthma with the exception of 1 patient who was atopic (Table I). All subjects were lifelong nonsmokers. Subjects with a history of respiratory tract infection within the previous 6 weeks or who had received immunotherapy within the previous 12 months were excluded. The study was approved by the Ethics Committee at the Laval Hospital, and written informed consent was obtained from all subjects before entry into the study.

Fiberoptic bronchoscopy and processing of tissues

Bronchial biopsy specimens were obtained from all patients as per the American Thoracic Society guidelines.¹⁹ Biopsies were performed on the patients with moderate-to-severe asthma on two separate occasions: at baseline and again after the 2-week course of oral corticosteroids. Specimens were obtained from the bronchial segmental divisions using alligator forceps (Olympus, Tokyo, Japan) and were blocked immediately by embedding the tissue in optimal cutting temperature medium and snap-freezing it in liquid nitrogen-cooled isopentane. Blocks were stored at -80°C until use.

Immunocytochemistry

For mAbs, the alkaline phosphatase/antialkaline phosphatase procedure was used as previously described.²⁰ For polyclonal mAbs, a biotinylated rabbit anti-goat antibody (1/500; Sigma Chemical, St Louis, Mo) was used and was developed by an alkaline phosphatase-conjugated streptavidin solution (1/100; Sigma Chemical). For all subjects, cryostat sections (6 µm) were cut from the frozen biopsy specimens, air-dried for 1 hour, fixed in an acetone/methanol (60:40) solution, and stained with the following antibodies: TGF β polyclonal antibody (1 µg/mL), mouse antihuman IL-11 mAb (20 µg/mL), and goat antihuman IL-17 from R&D systems (Minneapolis, Minn); mouse antihuman type I collagen and mouse antihuman type III collagen mAbs (5 µg/mL) from MediCorp Inc (Montreal, Quebec, Canada). An enhancement was performed for the IL-17 immunostaining by repeating the incuba-

tions with the secondary and tertiary antibodies. All of the slides were then developed in Fast Red (1 mg/mL; Sigma Chemical) and counterstained in hematoxylin. For negative control preparations, the primary antibody was replaced by TRIS-buffered saline solution or the irrelevant isotype and species-matched primary antibody.

Quantitation

Slides were counted blindly. Positive cells within the epithelium were counted, and the results were expressed as the percentage of positive epithelial cells on a 0.5-mm basement membrane length. Positive cells in the subepithelium were counted and expressed as the number of positive cells per square millimeter of submucosa. The extent of collagen staining in the subepithelium and the thickness of the collagen layer below the basement membrane were quantified with an image analysis system (Image Pro-Plus software, Media Cybernetics, Silver Spring, Md). These data are expressed as the mean of 3 measurements.

Statistical analysis

Significant differences among groups and conditions for International Certification Commission experiments were determined through use of the nonparametric Kruskal-Wallis test and subsequently through use of a Mann-Whitney *U* test. Correlation coefficients were obtained by Pearson's rank method with Bonferroni correction (Systat v7.0; SPSS, Chicago, Ill). In each case, a *P* value of less than .05 was considered statistically significant.

RESULTS

Expression of TGF- β

The expression of TGF- β was low in the bronchial submucosa of subjects in the control group. The immunoreactivity for TGF- β was significantly higher in the patients with mild asthma and the patients with moderate-to-severe asthma than in the controls ($P < .05$, Fig 1). Most of the TGF- β immunoreactivity was localized to the inflammatory cells in the subepithelial tissues. When we compared expression of TGF- β in the moderate-to-severe group before and after oral corticosteroids, no significant difference was found in the TGF- β immunoreactivity after oral corticosteroids.

Expression of IL-11

The expression of IL-11 in the subepithelium was similar in the controls and the subjects with mild asthma (40 ± 24 and 46 ± 12 positive cells/mm² of tissue, respectively). In turn, the immunoreactivity of IL-11 was significantly higher in the patients with moderate-to-severe asthma (111 ± 31 positive cells/mm² of tissue; $P \leq .05$ in comparison with controls and patients with mild asthma; Fig 2, A). After treatment with oral corticosteroids, the expression of IL-11 in the subepithelium of patients with moderate-to-severe asthma was decreased to levels (23 ± 11 positive cells/mm² of tissue) that were significantly lower than their baseline levels ($P < .005$). In the epithelium, higher levels of IL-11 were observed in the patients with moderate-to-severe asthma ($28.7\% \pm 9.3\%$ of positive epithelial cells/0.5 mm of basement membrane) than in the controls and the patients with mild asthma in whom the levels were low (0% and $2.5\% \pm 2.0\%$ of positive epithelial cells/0.5 mm of basement membrane for controls and patients with mild asthma, respectively; $P \leq$

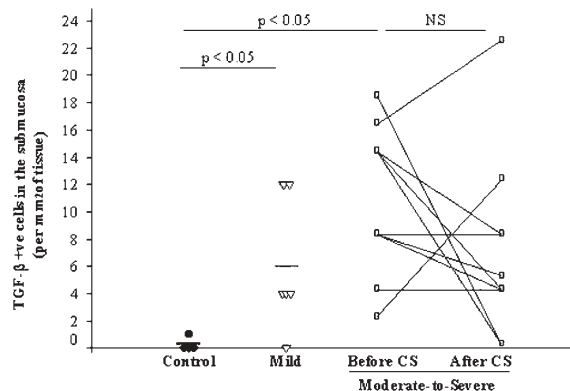


FIG 1. Comparison of TGF- β immunoreactivity in the bronchial submucosa of control subjects, patients with mild asthma, and patients with moderate-to-severe asthma (before and after the 2-week course of oral corticosteroids).

.001; Fig 2, B). The high epithelial expression of IL-11 in the patients with moderate-to-severe asthma was decreased after corticosteroid treatment ($P = .005$) to levels that were similar to those in the epithelium of patients with mild asthma ($4.5\% \pm 2.4\%$ of epithelial cells/0.5 mm of basement membrane; Fig 2, B).

Expression of IL-17

The immunoreactivity for IL-17 in the subepithelium of control subjects and patients with mild asthma was similar in both groups (31 ± 9 and 42 ± 9 positive cells/mm² of tissue for controls and patients with mild asthma, respectively, Fig 3, A). No staining was detected in the epithelial compartment for either group (Fig 3, B). In contrast, high levels of IL-17 were found both in the subepithelial (Fig 3, A) and epithelial (Fig 3, B) compartments of the patients with moderate-to-severe asthma (181 ± 53 positive cells/mm² of tissue and $79.4\% \pm 3.9\%$ of positive epithelial cells/0.5 mm of basement membrane, respectively). These levels were significantly different from those detected in the control and subjects with mild asthma ($P \leq .001$ for all). After a 2-week course of oral corticosteroids, IL-17 immunoreactivity decreased significantly in the subepithelium and the epithelium of the patients with moderate-to-severe asthma (29 ± 2 positive cells/mm² of tissue and $4.9\% \pm 1.6\%$ of positive epithelial cells/0.5 mm of basement membrane, respectively, Fig 3, A and B). These levels were significantly different from those measured before corticosteroid treatment ($P < .001$ in both subepithelium and epithelium) and were similar to the levels in the controls and patients with mild asthma.

Expression of collagens

Fig 4 shows results for type I collagen. The expression of collagen was higher in patients with mild asthma than in controls (scores: 1.56 ± 0.41 and 0.8 ± 0.4 for type I collagen, respectively; 7.56 ± 0.22 and 6.19 ± 0.07 for type III collagen, respectively); however, the differences were not statistically different. In contrast, both types of

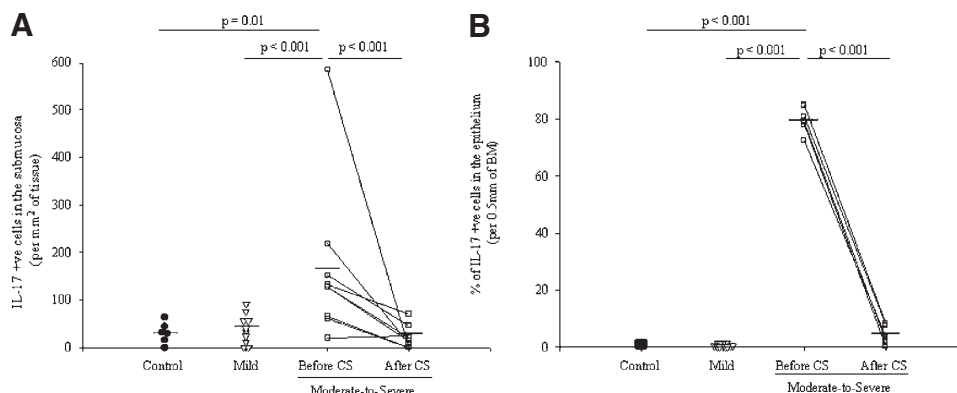


FIG 2. A, Comparison of IL-11 immunoreactivity in the bronchial submucosa of control subjects, patients with mild asthma, and patients with moderate-to-severe asthma (before and after the 2-week course of oral corticosteroids). B, Comparison of IL-11 immunoreactivity in the bronchial epithelium of control subjects, patients with mild asthma, and patients with moderate-to-severe asthma (before and after the 2-week course of oral corticosteroids).

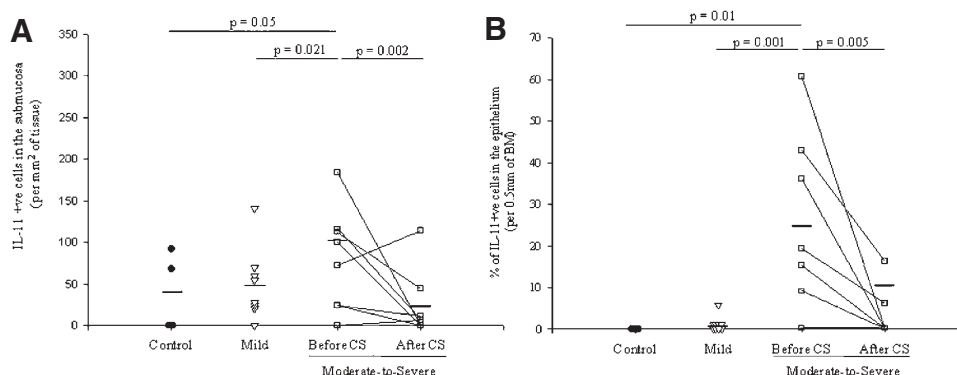


FIG 3. A, Comparison of IL-17 immunoreactivity in the bronchial submucosa of control subjects, patients with mild asthma, and patients with moderate-to-severe asthma (before and after the 2-week course of oral corticosteroids). B, Comparison of IL-17 immunoreactivity in the bronchial epithelium of control subjects, patients with mild asthma, and patients with moderate-to-severe asthma (before and after the 2-week course of oral corticosteroids).

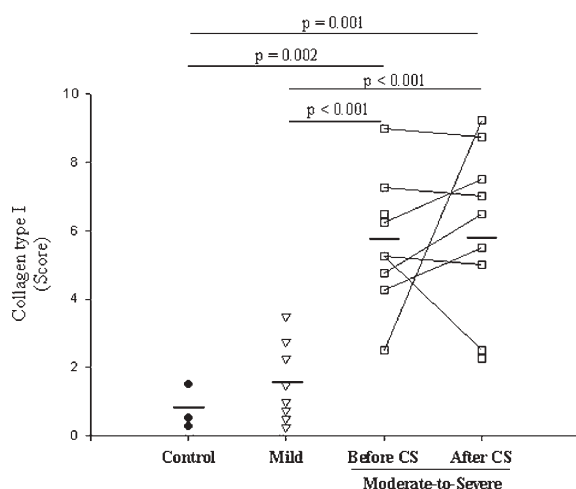


FIG 4. Immunoreactivity of type I collagen in the submucosa of control subjects, patients with mild asthma, and patients with moderate-to-severe asthma (before and after the 2-week course of oral corticosteroids).

collagen were significantly increased in patients with moderate-to-severe asthma (5.75 ± 0.56 and 8.53 ± 0.26 for type I and type III, respectively) compared with either patients with mild asthma ($P < .001$ and $P = .05$ for type I and type III, respectively) and controls ($P < .005$ and $P < .05$ for type I and type III, respectively). The 2-week course of corticosteroid treatment did not alter these levels significantly (5.80 ± 0.92 and 8.28 ± 0.25 for type I and type III, respectively).

DISCUSSION

In this study, we confirmed that bronchial biopsy specimens of patients with moderate-to-severe asthma are characterized by increased deposition of types I and III collagens compared with patients with mild asthma and normal controls. We also showed that there is preferential upregulation of the expression of profibrotic cytokines TGF- β , IL-11, and IL-17 in patients with moderate-to-severe asthma compared with patients with mild asthma and normal controls. As such, our data are the first to

demonstrate that as with TGF- β and IL-11, the immunoreactivity of IL-17 correlates with disease severity. In addition, we have shown that treatment with oral corticosteroids significantly reduces the expression of IL-11 and IL-17 in the airways of patients with moderate-to-severe asthma. In contrast, TGF- β expression was not altered by the 2-week course of oral corticosteroids.

Subepithelial fibrosis is an important feature of airway remodeling and has been extensively studied, because it is easy to identify and measure in bronchial biopsy specimens. Historically, subepithelial fibrosis was referred to as “thickening of the basement membrane,” until Jeffery²¹ demonstrated that these changes are related to deposition of fibrous tissue and extracellular proteins below the basement membrane. We and others have shown a correlation between the severity of asthma and the degree of subepithelial fibrosis.^{15,22}

The ECM is composed of a complex network of macromolecules, including collagen, elastin, fibronectin, tenascin, and proteoglycan. Collagen, in particular, is a major protein of the ECM. Interestingly, the subepithelial tissues of patients with asthma have been shown to contain significantly more type I and type III collagen than those of normal control subjects.²³ We have previously demonstrated a positive correlation between the expression of types I and III collagen and the severity of disease.¹⁵ A more recent publication from our group showed a similar association between proteoglycan deposition and increased airway responsiveness.²⁴ It should be noted, however, that the correlation between subepithelial fibrosis and disease severity in asthma is still a point of debate in the literature, because other groups have failed to observe these associations.²⁵ The disparities in the reports might be explained by the varying definitions of disease severity and differences in the methods used to detect extracellular proteins between the groups. In this study, we provide additional evidence to support the association between the extent of subepithelial fibrosis as measured by the staining of types I and III collagens and the disease severity in asthma.

TGF- β represents a profibrotic cytokine that is produced by a number of cells, including macrophages, epithelial cells, fibroblasts, and eosinophils. The expression of TGF- β in patients with asthma has varied among investigators, and whether its level correlates with disease severity is controversial.^{26,27} We have previously demonstrated an association between the level of expression of TGF- β and severity of disease; however, other groups did not observe this association.¹¹ In this study, we have confirmed that TGF- β expression is increased in patients with asthma compared with controls, and we also showed that the degree of expression is significantly higher in the patients with moderate-to-severe disease than in patients with mild asthma. Although we did not do any colocalization studies to detect the site of the TGF- β , earlier studies reported that most of the TGF- β expression in patients with asthma comes from eosinophils, which are increased in patients with asthma and likely correlate with disease severity.²⁸ Epithelial

cells might be an additional source of TGF- β ; however, we observed only minimal amounts of TGF- β in the epithelial cells in our biopsy specimens (data not shown). This might be explained by damage of epithelial cells after endobronchial biopsies.

IL-11 is a member of the IL-6 family of cytokines. It has been previously shown that IL-11 has fibrogenic potential in an animal model and is involved in the formation of subepithelial fibrosis and the local accumulation of fibroblasts, myofibroblasts, and smooth muscle cells. We have previously shown that though IL-11 is not increased in patients with mild asthma compared with controls, its expression is significantly higher in the patients with more severe disease, and we have confirmed this observation in this study.¹⁵

IL-17 is a newly described cytokine that has been identified as a T-cell product of the T_H2 type and is increased in patients with mild asthma compared with normal controls.¹⁶ In this study, we observed upregulation of the expression of this cytokine in patients with moderate-to-severe disease with a pattern similar to that of IL-11. Furthermore, the response of IL-17 to corticosteroids seems to be similar to IL-11 in that its expression was decreased significantly after treatment with oral steroids.

Although we did not do double-staining in this study to identify which cells express IL-11, IL-17, or TGF- β , we have reported previously the major cellular phenotypes that produce these cytokines in asthma. These profibrotic cytokines are produced in large part by inflammatory cells, such as eosinophils, as well as by structural cells, including epithelial cells.^{11,15}

The effects of corticosteroids on airway remodeling and subepithelial fibrosis have not been fully explained. In this study, we showed that a 2-week course of oral corticosteroids does not produce a decline in the expression for types I and III collagen. Fibroblasts seem to be resistant to steroids in terms of their ability to produce collagen of types I and III in response to IL-11 and IL-17.¹⁶ Although we have not measured the degree of subepithelial fibrosis by van Giemsa staining, our findings are consistent with the previous studies from our group and other groups that corticosteroids are not effective in reducing subepithelial fibrosis and inhibiting fibroblast function.²⁹ Fibroblast function is controlled by a number of mediators such as IL-11, IL-17, and TGF- β . The cytokines IL-11 and IL-17 do not seem to be the key factors that lead to the resistance of fibroblasts to corticosteroids, because their immunoreactivity was reduced after the 2-week course of oral corticosteroids. In contrast, the expression of TGF- β was not suppressed by treatment with oral corticosteroids. This finding might explain the persistent collagen production from fibroblasts in patients with severe asthma despite treatment with oral corticosteroids.

It is also important to note that the deposition of collagen is regulated by levels of matrix metalloproteinases, a family of enzymes that is responsible for cleaving protein constituents of the ECM. This degradation process is balanced by a number of specific inhibitors of matrix

metalloproteinases, including tissue inhibitor of metalloproteinases. In this study, we did not examine the expression of matrix metalloproteinases or tissue inhibitor of metalloproteinases, and it is conceivable that they play a role in producing the apparent insensitivity of collagen to corticosteroids.

In conclusion, despite the ability of corticosteroids to reduce airway inflammation and cytokine gene expression, they do not seem to inhibit fibroblast function, airway remodeling, and subepithelial fibrosis. These data emphasize the need in clinical practice for a therapy that will target fibroblast function. Recently, some reports have indicated that leukotriene receptor antagonists might have antifibrotic effects.³⁰ If this is true, further studies will be necessary to investigate the effect of leukotriene antagonists on fibroblast function and their impact on airway remodeling.

We thank Luce Trépanier for recruiting the subjects and Doris Cantin and Nathalie Pagé for their excellent technical assistance.

REFERENCES

- Redington AE, Howarth PH. Airway wall remodeling in asthma. *Thorax* 1997;52:310-2.
- Nelson HS, Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. J Allergy Clin Immunol 2003;111:215-25.
- Massagué J. TGF- β signal transduction. *Annu Rev Biochem* 1998;67:753-91.
- Border WA, Noble NA. Transforming growth factor β in tissue fibrosis. *N Engl J Med* 1994;331:1286-92.
- Grande JP. Role of transforming growth factor-beta in tissue injury and repair. *Proc Soc Exp Biol Med* 1997;214:27-40.
- Ignatz RA, Massagué J. Transforming growth factor- β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol Chem* 1986;261:4337-45.
- Kovacs EJ, Dipietro LA. Fibrogenic cytokines and connective tissue production. *FASEB J* 1994;8:854-61.
- Laurent GJ, Coker RK, Mcanulty RJ. TGF- β antibodies: a novel treatment for pulmonary fibrosis? *Thorax* 1993;48:953-4.
- Mcanulty RJ, Chambers RC, Laurent GJ. Regulation of fibroblast procollagen production. Transforming growth factor- β_1 induces prostaglandin E2 but not procollagen synthesis via a pertussis toxin-sensitive G-protein. *Biochem J* 1995;307:63-8.
- Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, et al. Transforming growth factor- β_1 in asthma. Measurement in bronchoalveolar lavage fluid. *Am J Respir Crit Care Med* 1997;156:642-7.
- Minshall EM, Leung DYM, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF- β_1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997;17:326-33.
- Elias JA, Zheng T, Einarsson O, Landry M, Trow T, Rebert N, et al. Epithelial interleukin-11. Regulation by cytokines, respiratory syncytial virus, and retinoic acid. *J Biol Chem* 1994;269:22261-8.
- Elias JA, Zheng T, Whiting NL, Trow TK, Merrill WW, Zitnik R, et al. IL-1 and transforming growth factor-beta regulation of fibroblast-derived IL-11. *J Immunol* 1994;152:2421-9.
- Tang W, Yang L, Yang YC, Leng SX, Elias JA. Transforming growth factor- β stimulates interleukin-11 transcription via complex activating protein-1-dependent pathways. *J Biol Chem* 1998;273:5506-13.
- Minshall E, Chakir J, Laviolette M, Molet S, Zhu Z, Olivenstein R, et al. IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils. *J Allergy Clin Immunol* 2000;105(2 Pt 1):232-8.
- Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Pagé N, et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol* 2001;108:430-8.
- Leung DY, Bloom JW. Update on glucocorticoid action and resistance. *J Allergy Clin Immunol* 2003;111:3-22.
- Anonymous. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am Rev Respir Dis* 1987;136:225-44.
- Workshop summary and guidelines: investigative use of bronchoscopy, lavage, and bronchial biopsies in asthma and other airway diseases. *J Allergy Clin Immunol* 1991;88:808-14.
- Ghaffar O, Laberge S, Jacobson MR, Lowhagen O, Rak S, Durham SR, et al. IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison with IL-4 expression and modulation by topical glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1997;17:17-24.
- Jeffery PK. Pathology of asthma. *Br Med Bull* 1992;48:23-39.
- Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;1:520-4.
- Chakir J, Laviolette M, Boutet M, Laliberté R, Dubé J, Boulet L-P. Lower airways remodeling in nonasthmatic subjects with allergic rhinitis. *Lab Invest* 1996;75:735-44.
- Huang J, Olivenstein R, Taha R, Hamid Q, Ludwig M. Enhanced proteoglycan deposition in the airway wall of atopic asthmatics. *Am J Respir Crit Care Med* 1999;160:725-9.
- Chu HW, Halliday JL, Martin RJ, Leung DYM, Szeffler SJ, Wenzel SE. Collagen deposition in large airways may not differentiate severe asthma from milder forms of the disease. *Am J Respir Crit Care Med* 1998;158:1936-44.
- Vignola AM, Chanez P, Chiappara G, Merendino A, Pace E, Rizzo A, et al. Transforming growth factor- β expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997;156:591-9.
- Hoshino M, Nakamura Y, Sim JJ. Expression of growth factors and remodeling of the airway wall in bronchial asthma. *Thorax* 1998;53:21-7.
- Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, et al. Transforming growth factor beta 1 (TGF beta 1) gene expression by eosinophils in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1996;15:404-9.
- Jeffery PK, Godfrey RW, Ädelroth E, Nelson F, Rogers A, Johansson SA. 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* 1992;145:890-9.
- Henderson WR Jr, Tang LO, Chu SJ, Tsao SM, Chiang GK, Jones F, et al. A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. *Am J Respir Crit Care Med* 2002;165:108-16.