Airways Remodeling Is a Distinctive Feature of Asthma and Is Related to Severity of Disease*

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Purpose: Airways remodeling, evaluated as the subepithelial layer thickness, was compared in asthmatic patients with that of healthy subjects, and was related to clinical grading of disease, presence of atopy, and length of asthmatic history.

Subjects and methods: Thirty-four patients with stable asthma (mean age±SD: 26.5±9.2 years; 10 female) treated with only inhaled β₂-agonists and eight healthy volunteers (mean age±SD: 24.6±2.5 years; four female) were recruited for the study. Twenty-seven of 34 asthmatics had atopy. Eleven patients had newly diagnosed conditions (duration of disease ≤ 1 year), nine patients had long asthmatic history (> 1 year and ≤ 10 years), and 14 had prolonged asthmatic history (> 10 years). Bronchial responsiveness to methacholine (M) was expressed as provocative concentration of M causing a 20% fall in FEV₁ (PC₂₀, mg/mL). Degree of asthma severity was assessed using a 0- to 12-point score based on symptoms, bronchodilator use, and daily peak expiratory flow variability over a 3-week period. Bronchoscopy and bronchial biopsy were performed successfully for all subjects; the subepithelial layer thickness, in biopsy samples, was measured from the base of bronchial epithelium to the outer limit of reticular lamina.

Results: In asthmatics, baseline FEV₁ values (percent of predicted) ranged from 75.7 to 137.0%, and PC₂₀ M ranged from 0.15 to 14.4 mg/mL. According to the asthma severity score, 14 asthmatics were classified as having mild disease, 14 as having moderate disease, and six as having severe disease. The mean values of subepithelial layer thickness were 12.4±3.3 μm (range, 6.8 to 22.1 μm) in asthmatics, and 4.4±0.5 μm (range, 3.8 to 5.2 μm) in healthy subjects (p<0.001). Subepithelial layer thickness of those with severe asthma differed significantly from that of patients with moderate and mild asthma (16.7±3.1 μm vs 12.1±2.7 μm and 10.8±2.4 μm, p<0.01 and p<0.003, respectively). Moreover, in asthmatics, degree of thickening was positively correlated to asthma severity score (Spearman rank correlation coefficient [rs]=0.581; p<0.001), and negatively correlated with baseline FEV₁ (rs=-0.553; p<0.001) and PC₂₀ M (rs=-0.510; p<0.01). No difference was found between degree of thickening observed in atopic asthmatics, compared with that of nonatopic asthmatics, or between degree of thickening in patients with different lengths of asthmatic history. Lastly, multiple regression analysis revealed that asthma severity score was the significant predictive factor for thickness of subepithelial layer.

Conclusions: We confirmed that airways remodeling is a very distinctive and characteristic pathologic finding of asthma. We also demonstrated that it is related to the clinical and functional severity of asthma, but not to atopy or length of asthmatic history.

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Key words: airways remodeling; asthma; subepithelial layer thickness

Abbreviations: F/M = female/male; M = methacholine; PC₂₀ M = provocative concentration of methacholine causing a 20% fall in FEV₁; PEF = peak expiratory flow; rs = Spearman rank correlation coefficient

Bronchial asthma, no matter how severe, is a chronic inflammatory disorder of the airways.¹ Chronic inflammation causes pathologic changes in the airways of asthmatic patients, markedly remodel-

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cantly related to airway inflammation cell markers, such as eosinophils, as well as to baseline airway patency and bronchial responsiveness to methacholine (M).\textsuperscript{7} Hence, airway remodeling in asthma may be a distinctive pathologic finding of great clinical and functional significance. In this regard, some studies have highlighted the differences between the thickness of the subepithelial layer in asthmatics and healthy control subjects.\textsuperscript{1,5-10} To date and to our knowledge, there are no data in the literature establishing a clear relationship among degree of subepithelial thickening, clinical grading of asthma, presence of atopy, and length of asthmatic history.

In this study, as a primary hypothesis, we ascertained whether the degree of subepithelial thickening in a large group of asthmatic patients treated with only inhaled \( \beta_2 \)-agonists was significantly different from that of a group of healthy subjects. Also, we wanted to investigate in the asthmatic patients if any relationship existed between the thickness degree of the subepithelial layer and clinical grading of disease. As an exploratory analysis, we evaluated the relationship between the thickness degree of the subepithelial layer and presence of atopy and length of asthmatic history.

**Materials and Methods**

**Subjects**

We studied a group of 34 patients (age range, 18 to 55 years) with bronchial asthma as defined by the American Thoracic Society\textsuperscript{11} in our outpatient clinic (Table 1). We included lifetime nonsmoking patients with no respiratory infection or spontaneous asthmatic relapses in the 4 weeks prior to study. Respiratory symptoms were controlled with inhaled \( \beta_2 \)-agonists on a daily basis or as required. Patients requiring theophylline, steroids, or sodium cromoglycate were excluded. Baseline FEV\(_1\) had to be >70% of predicted value and varied by <5%. Presence of atopy, assessed by skin prick tests to a standard battery of eight common inhalant allergens, was not a prerequisite for selection. Patients with pollen-related asthma were studied outside pollen exposure.

Degree of asthma severity was assessed by a slightly modified version of the asthma severity score proposed by Woolcock and Jenkins.\textsuperscript{12} Possible scores ranged from 0 to 12. Asthma severity score was based on symptoms, bronchodilator use, and daily peak expiratory flow (PEF) variability, measured during 3 weeks prior to M challenge test day. Briefly, patients’ scores were 0 to 4 for symptoms, ranging from no symptoms=0; symptoms less than once weekly or on exercise=1; symptoms less than daily or more than once weekly=2; daily symptoms without nocturnal asthma symptoms=3; waking at night=4. Bronchodilator use scores were 0 to 4, ranging from no use=0; less than once a week=1; more than daily=2; one to four times a day=3; more than four times a day=4. PEF variability was calculated according to the following formula: highest value-lowest/highest value\times100; scores were 0 to 4, ranging 6%=0, 6 to 10%=1, 10 to 15%=2, 15 to 25%=3, >25%=4. Patients with 0 to 5 scores were classified as having mild disease, 6 to 8 as having moderate disease, and 9 to 12 as having severe disease. For length of asthmatic history, we divided asthmatics into three groups: subjects with newly diagnosed asthma (duration of disease \( \leq 1 \) year) and subjects with long asthmatic history and with prolonged history of asthma (duration of disease >1 year and \( \leq 10 \) years, and >10 years, respectively).

The control group included eight healthy volunteers recruited among medical students and hospital staff (Table 1). They were lifetime nonsmokers and did not experience any acute respiratory illness in the 4 weeks prior to study. All subjects denied any personal or family history of allergy and/or respiratory disease, including asthma. Each subject gave informed, signed consent. Study protocol was approved by the Parma Hospital and University of Parma, Ethical Committee. Part of the asthmatic population was included in another study to investigate the relationship between bronchial responsiveness to distilled water and M and bronchial biopsy findings.\textsuperscript{7}

**M Challenge Test**

M challenge test was performed according to standardized procedure.\textsuperscript{13} Pulmonary function was measured by a dry spirometer connected to a computer for data analysis (Spiroflow; P.K. Morgan; Kent, UK). Each subject inhaled doubling increasing concentrations of M (0.03 to 64 mg/mL), nebulized by a dosimeter with an output of 9±0.3 μL per puff (Dosimeter MB3; Mefar; Brescia, Italy), until FEV\(_1\) was reduced by 20% from postsaline solution value. Bronchial response to M was expressed as the provocative concentration causing a 20% fall in FEV\(_1\) (PC\(_{20}\) in mg/mL), and was calculated by using the log-dose response curve.

**Bronchoscopy and Biopsy Sample Processing**

At least 3 days after M challenge test, each subject underwent bronchoscopy by flexible fiberoptic bronchoscope (Olympus; 1T10; Tokyo, Japan). Subjects were IM treated with atropine (0.5 mg) and diazepam (10 mg). Local anesthesia was then performed by inhalation of an aerosol solution of 2 mL of 2% lidocaine followed by the suctioning of a 20-ng tablet of tetracaine 15 min before bronchoscopy. Immediately before and after bronchoscopy, all subjects inhaled an aerosol of salbutamol (1.25 mg) and ipratropium bromide (0.25 mg). An additional aliquot of 2% lidocaine was applied into the larynx during bronchoscopy.\textsuperscript{7}

<table>
<thead>
<tr>
<th>Table 1—Subjects’ Characteristics</th>
<th>Mild Asthma</th>
<th>Moderate Asthma</th>
<th>Severe Asthma</th>
<th>Healthy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No.</td>
<td>14</td>
<td>14</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Sex, F/M</td>
<td>4/10</td>
<td>5/9</td>
<td>1/5</td>
<td>4/4</td>
</tr>
<tr>
<td>Atopy, yes/no</td>
<td>12/2</td>
<td>10/4</td>
<td>5/1</td>
<td>0/6</td>
</tr>
<tr>
<td>Age, yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>25.1</td>
<td>27.1</td>
<td>29.2</td>
<td>24.6</td>
</tr>
<tr>
<td>Range</td>
<td>18-45</td>
<td>18-55</td>
<td>18-47</td>
<td>22-29</td>
</tr>
<tr>
<td>Duration of disease, yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6.9</td>
<td>10.6</td>
<td>17.7</td>
<td>—</td>
</tr>
<tr>
<td>Range</td>
<td>1-30</td>
<td>0.5-37</td>
<td>1-34</td>
<td>—</td>
</tr>
<tr>
<td>FEV(_1), % predicted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>109.8</td>
<td>100.3</td>
<td>83.4(*)</td>
<td>108.1</td>
</tr>
<tr>
<td>Range</td>
<td>52-137</td>
<td>75-126</td>
<td>76-89</td>
<td>93-117</td>
</tr>
<tr>
<td>M PC(_{20}), mg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean</td>
<td>4.16</td>
<td>1.38</td>
<td>0.43(*)</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Range</td>
<td>0.31-14.4</td>
<td>0.17-6.9</td>
<td>0.15-3.68</td>
<td>—</td>
</tr>
</tbody>
</table>

\(*p<0.001\) vs mild.  
\(p<0.02\) vs moderate.
Biopsy procedure and processing were carried out as previously described.\textsuperscript{14} Several bronchial mucosa biopsy specimens (three to five samples) were obtained by alligator forceps at the carina of the right upper lobe, the opening of the right middle or lower lobe, and inside the lower lobe. During bronchoscopy, ECG and oxygen saturation were monitored continuously. Patients were discharged from the hospital when sedation values recovered to baseline. Bronchial specimens were fixed in periodate-lysine-parafomaldehyde (pH 7.4) for 3 h and then washed in a buffered cacodylate solution with 7% sucrose for 12 h. Each specimen was embedded in plastic (glycol methacrylate; JB4 Polyciences Inc; Newton, Mass) and was cut into sections of 1-µm thickness. Tissue sections were then examined under light microscope at ×1,000 magnification (Splan; Olympus). Sections were coded and blindly examined by a single investigator (A.P.). The thickness of “total” basement membrane was also assessed on two sections of the same biopsy sample (100 µm apart). Several estimates were made in each section stained with hematoxylin-eosin, along its length at 200-µm intervals, and values were averaged. Thickness was measured from the base of bronchial epithelium to the outer limit of its reticular lamina. Thus, measurements included true and reticular basement membrane. Final thickness measurement was obtained by averaging all measurements performed in each biopsy specimen.

Data Analysis

PC20 M values were log-transformed before analysis. FEV\textsubscript{1} values were expressed as percent of predicted value. Values were presented as mean±SD and as geometric mean±geometric SEM. Differences in numeric data between groups were analyzed by means of the nonparametric Kruskal-Wallis one-way analysis of variance, using the Mann-Whitney U test to assess the significance of differences between pairs of groups where analysis of variance showed statistically significant differences. Differences in qualitative data were analyzed by Fisher’s Exact Test. Relationships were estimated by the Spearman rank correlation coefficient (rs). Multiple regression analysis was performed to assess the contributions of age, sex, presence of atopy, duration of asthma, baseline FEV\textsubscript{1}, PC20 M, and asthma severity score to the subepithelial layer thickness. A p value <0.05 was considered significant.

The coefficient of repeatability, as described by Bland and Altman,\textsuperscript{15} was used to compare measurements performed on the two sections of the same biopsy specimen. At least three replicate measurements of morphometric parameters were performed by the same observer, and the intraobserver reproducibility was assessed with the coefficient of variation for repeated measurements.

Results

In asthmatic subjects, baseline FEV\textsubscript{1} values ranged from 75.7 to 137.0% (mean±SD: 101.2±16.5%). Moreover, bronchial responsiveness of asthmatics to M ranged from severe to very mild: PC\textsubscript{20} M values ranged from 0.15 to 14.4 mg/mL (mean±geometric SEM: 1.69±1.25 mg/mL). In healthy subjects, FEV\textsubscript{1} baseline values ranged between 93.0 and 117.0% (mean±SD: 108.1±8.4%) and bronchial responsiveness to M was not measurable.

According to the asthma severity score, 14 asthmatics (female/male [F/M]: 4/10) were classified as having mild disease (score range, 2 to 5), 14 (F/M: 4/10) were classified as having moderate disease (score range, 6 to 8), and six (F/M: 1/5) were classified as having severe disease (score range, 9 to 10). When the FEV\textsubscript{1} values of the three asthmatic groups were compared, patients with severe asthma had values significantly lower than patients with moderate and mild asthma (81.1±6.3% vs 99.4±14.7% and 109.6±14.4%, p<0.03 and p<0.002, respectively). In addition, in the entire asthmatic group, FEV\textsubscript{1} values were negatively correlated to asthma severity score (rs=-0.505; p<0.01). When the difference in PC\textsubscript{20} M values was computed, patients with severe asthma had PC\textsubscript{20} M values (0.43±1.58 mg/mL) significantly lower than those with mild asthma (4.16±1.38 mg/mL; p<0.01), but not lower than those with moderate asthma (1.38±1.41 mg/mL; p=0.075). Furthermore, PC\textsubscript{20} M values were negatively correlated to asthma severity score (rs=-0.584; p<0.001). No differences were found among the three asthmatic groups with respect to the duration of disease, atopy, gender, or age.

Bronchoscopy and bronchial biopsy were successfully performed in all subjects. The coefficient of repeatability between measurements performed on the two sections of each biopsy specimen was 1.12 µm of subepithelial layer thickness. The mean coefficient of variation for three repeated measurements by the same observer of subepithelial layer thickness was 4%. Subepithelial layer thickness ranged from 6.8 and 22.1 µm (mean±SD: 12.4±3.3 µm) for asthmatics, and 3.8 to 5.2 µm (mean±SD: 4.4±0.5 µm) for healthy subjects (p<0.001) (Fig 1). When the difference in subepithelial layer thickness was computed, the groups of asthmatic patients were statistically different (p<0.003). Pairwise comparisons between asthmatics showed also that subepithelial collagen thickness of patients with severe asthma differed significantly from that of patients with moderate and mild asthma (16.7±3.1 µm vs 12.1±2.7 µm and 10.8±2.4 µm, p<0.01 and p<0.003, respectively). Moreover, in all asthmatics, subepithelial collagen thickness was positively correlated to asthma severity score (rs=0.581; p<0.001) (Fig 2), and negatively correlated with baseline FEV\textsubscript{1} (rs=-0.553; p<0.001) and PC\textsubscript{20} M values (rs=-0.510; p<0.01).

Twenty-seven asthmatics (F/M: 6/21) were atopic, as judged by one or more positive responses to skin prick tests, while the remaining seven asthmatics (F/M: 4/3) were nonatopic. No difference was found between subepithelial layer thickness of the atopic group compared with that of the nonatopic group (11.9±2.7 µm vs 13.9±4.9 µm). In addition, atopic asthmatics were more strongly correlated to subepithelial layer thickness with asthma severity score (rs=0.638; p<0.001) (Fig 3).
Figure 1. Mean and individuals values of subepithelial layer thickness in patients with severe, moderate, and mild asthma, and healthy subjects. Number sign indicates p<0.001, healthy subjects vs all asthmatic patients.

Figure 3. Mean and individual values of subepithelial layer thickness in patients with newly diagnosed asthma (duration of disease ≤1 year), with long history (<1 year and ≥10 years), and with prolonged history of asthma (>10 years).

Asthmatics with single sensitization did not differ from those with multiple sensitizations when we measured their subepithelial layer thickness. In all asthmatics, the duration of disease ranged from 0.5 to 37 years. Eleven subjects had newly diagnosed asthma, nine subjects had long asthmatic history, and 14 had prolonged history of asthma. When we compared the thickness degree of the subepithelial layer of these three asthmatic groups, no differences were found (12.9±4.2 μm, 12.4±2.6 μm, and 11.9±3.1 μm, respectively) (Fig 3). Lastly, in all asthmatics, subepithelial layer thickness did not differ in men or women (12.4±3.7 μm vs 12.2±2.1 μm).

In all asthmatic patients, a multiple regression analysis was performed considering subepithelial layer thickness as the dependent variable, while age, sex, atopy, duration of asthma, baseline FEV1, PC20 M, and asthma severity score were treated as independent variables. Analysis results indicated a significant contribution of asthma severity score to the subepithelial layer thickness, but not age, sex, atopy, duration of asthma, baseline FEV1, and PC20 M. More specifically, the overall adjusted coefficient of multiple regression for prediction of subepithelial layer thickness by these examined variables was 0.313 (p<0.001), and partial regression coefficient for asthma severity score was 0.837 (p<0.001).

Conclusions

We found a clear cutoff between the thickness of the subepithelial layer of asthmatics compared with that of healthy subjects. Thus, we confirmed that airway remodeling is a very distinctive and characteristic pathologic finding of bronchial asthma. Moreover, we demonstrated that in a large group of asthmatic patients treated with only inhaled β2-agonists on demand or on a regular basis, the degree of subepithelial layer thickness is highly related to clinical severity of asthma. However, we found no relationship between the subepithelial layer thickness degree and the presence of atopy or length of asthmatic history. Lastly, multiple regression analysis revealed that a clinical and functional severity score of asthma was the most important predicting factor for the subepithelial layer thickness, while age, sex, atopy, duration of asthma, baseline FEV1, or PC20 M were not significant factors influencing this finding.

Our results are in agreement with previous data, confirming that airway remodeling, due to
subepithelial layer thickness, clearly differentiates healthy from asthmatic subjects and should be considered as a peculiar characteristic of asthma. Interestingly, we found no overlap between asthmatic and healthy subjects (Fig 1). Moreover, we showed that the range of subepithelial layer thickness was more extensive than previously reported. Selection criteria of asthmatic patients might explain this difference. Previous studies included asthmatics treated with inhaled corticosteroids,\(^4,10\) asthmatics with small variations in disease severity,\(^9\) or patients older than 60 years.\(^8\) In our study, we recruited a large group of asthmatic patients ranging in age from 18 to 55 years, with a wide range of baseline airway patency and bronchial responsiveness to M and different clinical grading of disease. The diffuse thickening and hyalinization of the reticular layer beneath the lamina reticularis has long been recognized as a characteristic pathologic change occurring in asthma\(^16-18\) and may lead to major airway remodeling,\(^2\) while, on the other hand, in COPD, and in other chronic inflammatory diseases of the airways, such as bronchiectasis and tuberculosis,\(^19\) there may be only a focal and variable thickening of subepithelial layer. Subepithelial collagen thickness is due to an extensive deposition of interstitial proteins—including collagen types III and V, along with fibronectin in the subepithelial layer—and has previously been associated with the proliferation of subepithelial myofibroblasts.\(^6\) Furthermore, the negative correlation between intraepithelial eosinophils and degree of subepithelial layer thickness supports the hypothesis that inflammatory changes in the epithelium may also play an important role in initiating and sustaining this tissue repair process.\(^7\)

We provided strong evidence that in asthma, subepithelial collagen thickness is strictly linked to clinical grading of the disease. Past studies did not establish any relationship between degree of airway remodeling and clinical severity of asthma, as measured by only inhaled bronchodilator use\(^4\) or by symptoms score.\(^10\) Various clinical severity scores have been developed to quantify clinical grading of asthma, though many of these have not been completely validated.\(^20\) We used the Woolcock and Jenkins\(^12\) score, which is a 0 to 12 score based on symptoms, bronchodilator use, and daily PEF variability over 3 months. We reduced the observation period from 3 months to 3 weeks, since compliance and accuracy of daily PEF self-assessment for relatively long periods are generally poor.\(^21\) It has been suggested that this remodeling of the airways may have important functional and clinical significance.\(^22\) Also, its contribution to the presence of persistent bronchial hyperresponsiveness has been hypothesized in simulated models of asthmatic airways\(^23,24\) and recently confirmed by the close correlation between PC\(_{20}\) M and the degree of thickening.\(^7\) Indeed, the link between subepithelial layer thickness and clinical characteristics in asthma is confirmed by the thickness reduction that is associated with a clinical improvement of disease after a prolonged period of stimulus avoidance in isocyanate-sensitive asthmatics\(^25\) and following treatment with inhaled steroids in patients with mild asthma.\(^26,27\)

In this study, no relationship was found when the degree of subepithelial layer thickness of the atopic group was compared with that of the nonatopic group. To date and to our knowledge, no published data exist on the relationship between atopy and subepithelial layer thickness in asthma. However, with respect to number or type of airway inflammatory cells, no difference was observed in biopsy samples from atopic asthmatics when compared with those of nonatopic asthmatics.\(^28\) We did not find any relationship between the length of asthmatic history and airway remodeling. Similarly, in a small group of asthmatics, no correlation was found between duration of asthma and subepithelial layer thickness.\(^4\) The thickest subepithelial layer observed in our patients was obtained, interestingly enough, from a newly diagnosed asthmatic subject with severe grading of disease. This finding suggests that subepithelial collagen deposition is a very early change that occurs in asthma. Furthermore, airway remodeling could be a phenomenon caused by acute and repeated exposures to risk factors that may precede the clinical manifestations of asthma.\(^5\) Indeed, in a murine model of allergen-induced airway inflammation, after repeated challenges performed over a period of several weeks, the reticular layer beneath the basement membrane of the airway epithelium showed fibrosis, reproducing the pathologic feature of human asthma.\(^29\)

In conclusion, our results showed that airway remodeling clearly differentiates healthy from asthmatic airways and is strictly related to the severity of disease. Moreover, it seems that subepithelial layer thickness could be a very early pathologic phenomenon that may precede the clinical manifestations of disease.

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REFERENCES
1 National Institutes of Health, National Heart, Lung, and Blood Institute. Global strategy for asthma management and


8 Sobonya ES. Quantitative structural alterations in long-standing allergic asthma. Am Rev Respir Dis 1984; 130:289-92


11 American Thoracic Society. Standards for diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am Rev Respir Dis 1987; 136:225-44


22 Macklem PT. A hypothesis linking bronchial hyperreactivity and airway inflammation: implication for therapy. Ann Allergy 1990; 64:113-16


