

Epithelial Damage and Angiogenesis in the Airways of Children with Asthma

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Rationale: Airway remodeling and inflammation are characteristic features of adult asthma that are still poorly investigated in childhood asthma.

Objectives: To examine epithelial and vascular changes as well as the inflammatory response in airways of children with asthma.

Methods: We analyzed bronchial biopsies obtained from 44 children undergoing bronchoscopy for appropriate clinical indications other than asthma: 17 with mild/moderate asthma (aged 2–15 yr), 12 with atopy without asthma (1–11 yr), and 15 control children without atopy or asthma (1–14 yr). By histochemistry and immunohistochemistry, we quantified epithelial loss, basement membrane thickness, number of vessels, and inflammatory cells in subepithelium.

Results: Epithelial loss and basement membrane thickness were increased in children with asthma compared with control subjects ($p = 0.005$ and $p = 0.0002$, respectively) and atopic children ($p = 0.002$ and $p = 0.005$, respectively). The number of vessels and eosinophils was increased not only in asthmatic children ($p = 0.03$ and $p = 0.0002$, respectively) but also in atopic children without asthma ($p = 0.03$ and $p = 0.008$, respectively) compared with control subjects. When we stratified the analysis according to age, we observed that children with asthma younger than 6 yr had increased epithelial loss, basement membrane thickening, and eosinophilia compared with control subjects of the same age.

Conclusions: Epithelial damage and basement membrane thickening, which are pathologic features characteristic of adult asthma, are present even in childhood asthma. Other changes, such as airway eosinophilia and angiogenesis, were also observed in atopic children without asthma. These observations suggest that pathologic changes occur early in the natural history of asthma and emphasize the concept that some of these lesions may characterize atopy even in the absence of asthmatic symptoms.

Keywords: angiogenesis; basement membrane thickening; epithelial loss; pediatric asthma

Bronchial asthma has become an increasing concern for public health, especially in industrialized countries. The prevalence of asthma has increased significantly, particularly among children, and it is now the most frequent chronic medical condition in the pediatric age group (1–3).

Despite intensive research in recent years, the pathogenetic mechanisms of asthma are still poorly understood. It is well known that pathologic changes include both airway inflamma-

tion and remodeling, which may lead to thickening of the airway wall, therefore playing an important role in the pathophysiology of the disease (4). Although airway eosinophilia has been consistently reported in adults with asthma, the few pathologic studies performed in children reported controversial results (5–9). In addition, the majority of those studies were performed in children with severe asthma (6–8). Unavoidably, these children were treated with high-dose antiinflammatory therapy and this may have influenced the pathologic findings, in particular by reducing the number of eosinophils. Therefore, whether airway eosinophilia is present in children with asthma still remains to be elucidated.

Even less is known about the different components of airway remodeling in childhood asthma. Among these components, only reticular basement membrane thickness has been extensively investigated. Indeed, the pioneering qualitative observations of a thickened reticular basement membrane (9, 10) have been confirmed in quantitative studies performed on bronchial biopsies of children with mild to severe asthma (5–7, 11), suggesting that reticular basement membrane thickening is a pathologic hallmark that is present at all stages of disease severity. By contrast, other structural changes, such as epithelial loss and angiogenesis, have never been investigated in children. In particular, damage to the airway surface, resulting in epithelial loss at histologic analysis, has often been described in adults with asthma (12, 13), but only indirect evidence has been reported in childhood asthma (7, 14). Moreover, remodeling of the airway vascular bed, with new vessel formation and dilatation of preexisting vessels, is also a common feature of adult asthma that has never been investigated in children (15–18).

Hence, we performed this study to quantify new components of airway remodeling, such as epithelial damage and angiogenesis in childhood asthma. Moreover, we also examined reticular basement membrane thickness as well as different inflammatory cells infiltrating the airway wall. Toward this aim, among children undergoing bronchoscopy for appropriate clinical indications, we recruited the following three groups of subjects: a group of children with mild/moderate asthma (not influenced by high-dose antiinflammatory therapy), a group of atopic children without asthma, and a group of control children with no atopy or asthma.

Preliminary results of this study have been previously reported in form of abstract (19).

METHODS

Subjects

We examined 44 children who had undergone fiberoptic bronchoscopy for appropriate clinical indications other than asthma (20, 21). None of these children were included in our previous report in childhood asthma (5). The study population included the following three groups: 17 children with mild/moderate asthma (aged 2–15 yr), 12 atopic children without asthma (aged 1–11 yr), and 15 control children without asthma or atopy (aged 1–14 yr). Children with asthma underwent bronchoscopy for stridor ($n = 2$), recurrent pneumonia ($n = 8$), or chronic

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cough ($n = 7$). Atopic nonasthmatic children underwent bronchoscopy for stridor ($n = 3$), recurrent pneumonia ($n = 3$), chronic cough ($n = 4$), tracheomalacia ($n = 1$), or obstructive sleep apnea ($n = 1$). Control children underwent bronchoscopy for stridor ($n = 1$), recurrent pneumonia ($n = 6$), chronic cough ($n = 6$), tracheomalacia ($n = 1$), or laryngomalacia ($n = 1$).

Asthma was diagnosed when the child had repeated episodes of wheezing, breathlessness, and cough (particularly if associated with nocturnal and early-morning symptoms) that were responsive to bronchodilators (5, 22, 23). Presence and reversibility of episodic symptoms were assessed by parental reports and confirmed by the child's pediatrician. The presence of atopy was defined by an increase in total (paper radioimmunosorbent test [PRIST]) or specific (radioallergosorbent test [RAST]) IgE. All children in the three groups underwent PRIST, RAST, and routine blood tests, whereas spirometry was performed only in children who were able to cooperate with the test. The study was performed according to the Declaration of Helsinki and was approved by the ethics committee of Padova City Hospital. Informed consent was obtained from the children's parents. Details on asthma diagnosis, atopic status, and functional testing are included in the online supplement.

Sample Processing and Analysis

Bronchoscopy and bronchial biopsy processing were performed as previously described (5, 24) (*see* online supplement). Biopsies were considered suitable for examination when there was at least 1.0 mm of basement membrane length and 0.1 mm² of subepithelial area.

All morphometric measurements were performed using an image analysis system. In particular, analysis of epithelial loss and reticular basement membrane thickness was performed on sections stained with hematoxylin-eosin. For quantification of epithelial loss, we measured the length of incomplete epithelium and expressed it as a percentage of total epithelial length (*see* online supplement).

Vessels were assessed by immunohistochemistry using a monoclonal antibody anti-CD31. Number of vessels and vessel area were quantified in the area 100 μ m beneath the reticular basement membrane, as previously described (25), and expressed as number of vessels/mm² of examined subepithelium and as the percentage of area occupied by vessels over total area examined. The number of vessels expressing the vascular endothelial growth factor (VEGF) was assessed by immunohistochemistry in the subepithelium and expressed as number of positive vessels/mm² of examined subepithelium. VEGF expression was also examined in the epithelium using a semiquantitative score (ranging from 0 to 3). Finally, eosinophils, neutrophils, mast cells, macrophages, and CD4⁺ T lymphocytes were assessed in the subepithelium by immunohistochemistry, as previously described (5), and expressed as number of positive cells/mm² of examined subepithelium. Details on immunohistochemical, morphologic, and statistical analysis are included in the online supplement.

RESULTS

Clinical Findings

The characteristics of the children studied are shown in Table 1. The three groups of children were similar with regard to age. The bronchoscopy procedure was well tolerated by all children,

and no complications were encountered. Pulmonary function testing was successfully performed in 11 of 17 children with asthma, 6 of 12 atopic children without asthma, and 5 of 15 control subjects. FEV₁ values were significantly lower in children with asthma as compared with both atopic children and control children ($p = 0.03$ and $p = 0.01$, respectively).

In the children with asthma with an FEV₁ value less than 80% predicted, the mean response to bronchodilator was 14%, ranging from 12 to 16% of baseline values. At recruitment, the average duration of symptoms in children with asthma was 3.3 yr, ranging from 7 mo to 10 yr. In some of these children, symptoms were sometimes (but not exclusively) triggered by a cold or flu episode. Twelve of seventeen children with asthma had mild asthma and were only treated with inhaled salbutamol when needed. The remaining five children had moderate asthma and were treated regularly with combined salmeterol/fluticasone (50/100 μ g twice daily in four children and 25/125 μ g twice daily in one child). All children with recurrent pneumonia ($n = 17$) were being treated with antibiotics.

In the group of atopic nonasthmatic children, asthma was excluded because these children never had episodes of wheezing, breathlessness, or cough that were responsive to bronchodilators. Indeed, the four children with chronic cough received therapy with bronchodilators and corticosteroids for 2 wk without clinical benefit. Moreover, the two atopic nonasthmatic children who were able to perform exercise challenge to exclude a diagnosis of asthma had an FEV₁ fall after exercise of less than 10%. Atopic dermatitis was present, at the time of the study, in one of the atopic nonasthmatic children, whereas two others were affected in the first year of life but had no clinical manifestations thereafter. Details on atopic nonasthmatic children and PRIST and RAST results are reported in the online supplement.

Biopsy Findings

Nine children who were initially recruited to the study were subsequently excluded because their biopsies were not suitable for analysis (three children with asthma, one atopic nonasthmatic child, and five control children). We could perform all morphometric measurements in the remaining 44 children, except quantification of subepithelial vessels in one atopic nonasthmatic child.

Analysis of variance with a Kruskal-Wallis rank test showed that the three groups of children differed significantly with respect to percentage of epithelial loss ($p = 0.002$), thickness of the reticular basement membrane ($p = 0.0002$), and number of subepithelial vessels ($p = 0.041$) and eosinophils ($p = 0.004$).

The percentage of epithelial loss and the reticular basement membrane thickness were increased in children with asthma when compared with both control subjects ($p = 0.005$ and $p = 0.0002$, respectively) and atopic children without asthma ($p = 0.002$ and $p = 0.005$, respectively; Figures 1A, 1B, and 2). Atopic

TABLE 1. CLINICAL CHARACTERISTICS OF CHILDREN WITH ASTHMA, ATOPIC CHILDREN, AND CONTROL CHILDREN

	Children with Asthma	Atopic Children	Control Children
Number and sex, M/F	5 M/12 F	8 M/4 F	8 M/7 F
Age, median (range), yr	5 (2-15)	4 (1-11)	4 (1-14)
Symptom duration, median (range), yr	3.3 (7 mo-10 yr)	—	—
Atopy, n	7/17	12/12	0/15
FEV ₁ , mean \pm SE, % predicted	83 \pm 4*	100 \pm 6	102 \pm 5
FEV ₁ /FVC, mean \pm SE, %	88 \pm 2	90 \pm 3	91 \pm 3

Definition of abbreviations: F = female; M = male.

* $p = 0.03$ as compared with atopic children, and $p = 0.01$ as compared with control children.

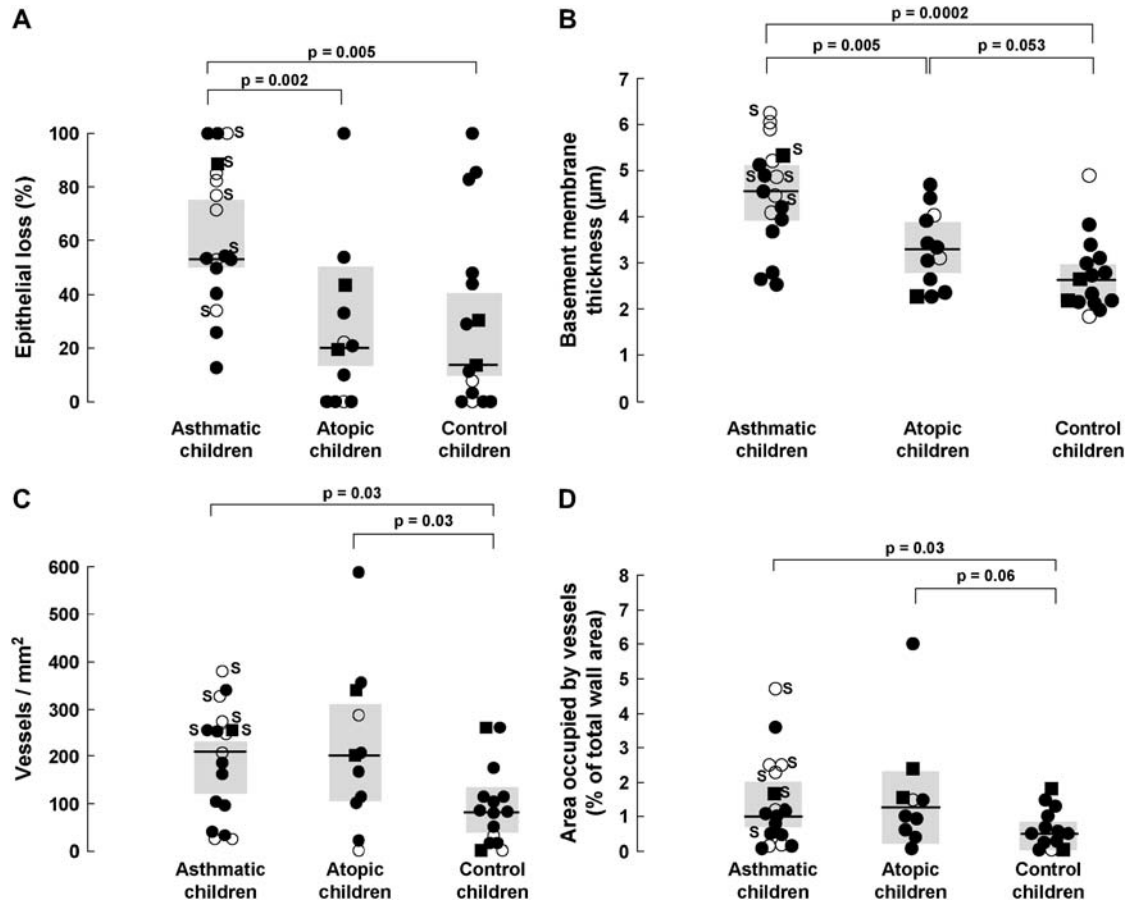


Figure 1. Individual values for (A) epithelial loss, (B) reticular basement membrane thickness, (C) subepithelial vessels, and (D) area occupied by vessels in bronchial biopsies. p values of Kruskal-Wallis rank test: $p = 0.002$ for epithelial loss; $p = 0.0002$ for reticular basement membrane thickness; $p = 0.041$ for number of subepithelial vessels. Filled squares indicate children < 3 yr; filled circles indicate children ≥ 3 yr and ≤ 6 yr; open circles indicate children > 6 yr. "S" indicates children with asthma who were treated with inhaled steroids. Horizontal bars represent median values, whereas boxes indicate 95% confidence intervals.

children without asthma showed a trend toward an increased reticular basement membrane thickness as compared with control children ($p = 0.053$), whereas the percentage of epithelial loss was similar in these two groups of subjects (Figures 1A and 1B). The epithelial damage we observed was mainly due to loss of the columnar layer, whereas loss of both basal and columnar cells was a rare occurrence, present in few subjects (four children with asthma, two atopic nonasthmatic children, and three control subjects) and involving only a small percentage of the whole epithelium (on average, 8.3%; ranging from 1.7 to 16.6%). The significant difference we observed in epithelial loss was confirmed either when we included the completely destroyed epithelium or when we considered the epithelium lacking columnar cells only.

The number of vessels, the percentage of area occupied by vessels, and the number of eosinophils in the subepithelium were increased in children with asthma when compared with control children ($p = 0.03$, $p = 0.03$, and $p = 0.0002$, respectively), whereas atopic children without asthma had values similar to those of children with asthma (Figures 1C, 1D, and 2; Table 2). When compared with control children, atopic children without asthma had increased numbers of subepithelial vessels ($p = 0.03$) and eosinophils ($p = 0.008$) as well as an increased percentage of vessel area; however, the last comparison did not reach the level of statistical significance ($p = 0.06$) (Figures 1C and 1D;

Table 2). The mean area of vessels was similar in children with asthma (median, 63; range, 22–126 μm^2), atopic children (median, 56; range, 28–102 μm^2), and control children (median, 55; range, 12–135 μm^2). Similarly, no significant differences were observed in the number of VEGF⁺ vessels among children with asthma (median, 20; range, 0–62 vessels/ mm^2), atopic nonasthmatic children (median, 25; range, 0–70 vessels/ mm^2), and control children (median, 14; range, 0–77 vessels/ mm^2). When we examined VEGF expression in intact epithelium and in damaged epithelium (where only basal cells were present), we found no significant differences among children with asthma (median, 0.9; range, 0.2–2.1; and median, 0.5; range, 0–1.9), atopic nonasthmatic children (median, 1.1; range, 0.3–1.9; and median, 1.0; range, 0.0–2.0), and control children (median, 0.8; range, 0.0–1.8; and median, 0.4; range, 0.0–1.5).

No significant differences were observed among the three groups of children examined in the number of neutrophils, mast cells, CD4⁺ T lymphocytes, and macrophages (Table 2).

When we split our population into those younger and those older than 6 yr of age, we found that children with asthma younger than 6 yr ($n = 10$) had an increased reticular basement membrane thickness, an increased epithelial loss, and an increased number of eosinophils as compared with control children of the same age ($n = 13$) (Table 3). As for number of vessels, although there was an increase in children with asthma

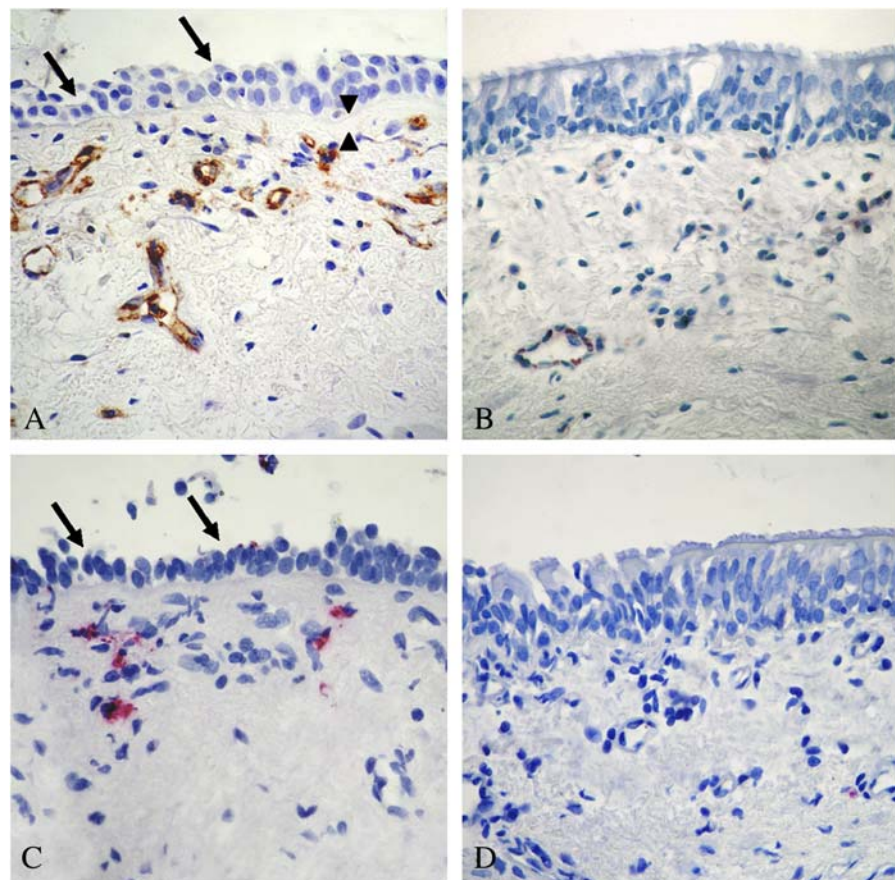


Figure 2. Bronchial biopsy sections from (A and C) a child with asthma and (B and D) a control child showing increased number of subepithelial vessels (A, brown) and eosinophils (C, red) in the child with asthma. Arrows indicate epithelial loss (A and C), whereas arrowheads indicate reticular basement membrane thickening (A). Immunostaining with monoclonal antibody anti-CD31 (A and B) and anti-EG2 (C and D). Original magnification, $\times 630$.

younger than 6 yr as compared with control subjects, it was not statistically significant (Table 3). In children with asthma younger than 6 yr, the percentage of epithelial loss (median, 53; range, 12–100%, vs. median, 77; range, 34–100%), the number of subepithelial vessels (median, 174; range, 31–339, vs. median, 245; range 25–377 vessels/mm²), and the number of eosinophils (median, 33; range, 8–90, vs. median, 85; range, 23–216 cells/mm²) were similar to those of children with asthma older than 6 yr. Conversely, reticular basement membrane thickness was increased in older children with asthma as compared with younger ones (median, 5.2; range, 4.1–6.2, vs. median, 4.1; range, 2.5–5.3 μ m; $p = 0.03$).

The mean length of epithelium examined per patient was 1.9 mm, ranging from 1.1 to 5.5 mm. The mean total area of subepithelium examined for each child was 0.18 mm², ranging from 0.11 to 0.5 mm². The coefficient of variation for repeated measurements (intraobserver variability) was 4% for the number of subepithelial vessels, 5.3% for the area occupied by vessels, 4.1% for epithelial loss, and 7% for reticular basement mem-

brane. For inflammatory cells, the coefficient of variation ranged from 6 to 14%.

When all children were considered together, the values of FEV₁ were inversely correlated with both the number of subepithelial vessels ($r = -0.43$, $p = 0.046$) and the area occupied by vessels ($r = -0.47$, $p = 0.037$). This last correlation remained significant when control subjects were excluded from the analysis. Moreover, the number of subepithelial eosinophils was positively correlated with both thickness of the reticular basement membrane ($r = 0.36$, $p = 0.02$) and degree of epithelial loss ($r = 0.40$, $p = 0.01$). This last correlation remained significant when control subjects were excluded from the analysis ($r = 0.38$, $p = 0.05$). No other significant correlations were observed between morphometric measurements and functional data.

DISCUSSION

This study shows that epithelial damage and angiogenesis, which are important components of airway remodeling in adult asthma,

TABLE 2. CELLULAR COUNTS IN THE SUBEPITHELIUM

	Children with Asthma	Atopic Children	Control Children
Eosinophils, cells/mm ²	38 (8–216)*	28 (0–207)†	8 (0–845)
CD4 ⁺ T lymphocytes, cells/mm ²	135 (0–1,191)	334 (85–662)	173 (0–524)
Macrophages, cells/mm ²	77 (0–225)	145 (19–345)	62 (0–535)
Neutrophils, cells/mm ²	100 (0–322)	127 (14–507)	92 (0–222)
Mast cells, cells/mm ²	56 (0–282)	100 (0–394)	73 (6–322)

Values are expressed as median (range).

* $p = 0.0002$ as compared with control children.

† $p = 0.008$ as compared with control children.

TABLE 3. STRUCTURAL CHANGES IN CHILDREN WITH ASTHMA AND CONTROL CHILDREN YOUNGER THAN 6 YEARS

	Children with Asthma ≤ 6 yr	Control Children ≤ 6 yr	p Values
Epithelial loss, %	53 (12–100)	29 (0–100)	0.05
Basement membrane thickness, μm	4.1 (2.5–5.3)	2.7 (2.0–3.8)	0.004
Vessels/mm ²	174 (31–339)	85 (0–260)	NS
Eosinophils/mm ²	33 (8–90)	7 (0–845)	0.004

Definition of abbreviation: NS = not significant.
Values are expressed as median and range.

are already present in children with mild/moderate asthma. These new pathologic findings together with those previously described (e.g., reticular basement membrane thickening and eosinophilia) improve our knowledge of airway pathology in childhood asthma.

Epithelial loss, although somewhat controversial (26), has been described as a typical feature of adult asthma (12, 13). Conversely, it has not been thoroughly investigated in childhood asthma and only indirect evidence of epithelial damage has so far been provided (7, 14). Indeed, Marguet and coworkers observed an increased percentage of epithelial cells in bronchoalveolar lavage from children with asthma as compared with control subjects (14), whereas in bronchial biopsies, Fedorov and coworkers reported an overexpression of epidermal growth factor receptor (EGFR), a marker of epithelial damage (7). Because this epithelial damage was not associated with an adequate proliferative response, the authors suggested that the epithelium of children with asthma may be unable to repair itself after injury (7). Our study, which, to the best of our knowledge, is the first to provide direct evidence of epithelial loss in children with asthma, confirms and extends those previous findings. We are well aware that epithelial loss in asthma is a matter of debate and it has been sometimes considered artifactual due to biopsy sampling procedures (26). On the other hand, it has been hypothesized that the epithelium could be more friable in asthma and therefore more liable to become detached during the biopsy process. In our study, biopsies from all groups were collected and processed using the same procedures. It is therefore unlikely that epithelial loss is an artifact due to biopsy procedures only, because it would have been evenly distributed in the three groups. The fact that more loss was found in children with asthma leads us to believe that it is due to a greater fragility of the epithelium and this epithelial instability may have an important pathogenetic role in asthma (27). Indeed, a damaged epithelium may trigger all the events leading to airway remodeling by releasing mitotic and fibrogenic growth factors (27), which may promote smooth muscle proliferation, angiogenesis, and increased collagen deposition, resulting in reticular basement membrane thickening. In support of this hypothesis, in addition to epithelial damage, we also observed reticular basement membrane thickening and increased vascularity in children with asthma, thereby highlighting the concept that an abnormal functioning of the epithelial–mesenchymal unit is a crucial pathologic hallmark of asthma (27).

Remodeling of the airway vascular bed has been described in adult asthma, with several quantitative studies reporting an increase in both total number of vessels and vascular area (15–18). Our study has demonstrated that an increased number of vessels and an increased vascular area are also present in children with asthma. Moreover, evidence of new vessel formation was present even in atopic children without asthma. It is important to highlight that bronchial vessels may act as the portal of entry for inflammatory cells to the airway wall and therefore may

contribute to chronic inflammation. This view is supported by the observations that significant airway eosinophilia was present not only in children with asthma but also in atopic children without asthma in both the present study and our previous one (5). Interestingly, even in atopic children without asthma, we observed a trend toward an increased reticular basement membrane thickness. Altogether, these findings are consistent with the emerging concept that, in atopic subjects, lower airway remodeling and inflammation are present even in the absence of specific bronchial symptoms (28). On the other hand, we should acknowledge that, because allergic sensitization plays an important role in the development of asthma early in life, it is possible that some of the atopic children in our study will eventually develop asthma (29). In this context, it is tempting to speculate that pathologic changes may precede the future development of asthma, as suggested by Pohunek and coworkers who showed thickening of the reticular basement membrane in young children up to 4 yr before asthma diagnosis (30). However, whether pathologic changes in the airways occur before the onset of asthma symptoms is still a matter of debate. In fact, Saglani and coworkers showed that wheezing infants (younger than 2 yr) with reversible airflow obstruction had no evidence of reticular basement membrane thickening or eosinophilia (31). In light of these observations, we performed an age-stratified analysis in our study and we observed that airway remodeling and eosinophilic inflammation were already present in the group of preschool children (age range, 2–6 yr). Taken together, these findings support the hypothesis that pathologic changes, although not present at birth, are early events in the natural history of asthma that appear in the preschool period, when both the respiratory and the immune system are completing their development (32).

Of interest in our study, when we investigated the inflammatory response, we found an increased number of eosinophils in children with asthma, whereas no differences were observed in other inflammatory cells among the three groups of children examined. These results confirm the findings of our own previous study performed in a different population of children with asthma (5) as well as prior observations in induced sputum (33, 34). However, our findings contrast with previous reports showing lack of eosinophilia in bronchial biopsies of children with asthma (6–8, 35). A possible explanation for this discrepancy is that children in the latter studies were undergoing treatment with high-dose antiinflammatory agents, which may have substantially reduced eosinophil numbers, whereas most children in our study had not received such therapy. Moreover, our observation that inflammatory cells other than eosinophils were not increased in bronchial biopsies of children with asthma is in agreement with previous studies performed in children (5, 6) and it is not surprising even when compared with studies in adults. Indeed, although it has been proposed that neutrophils, mast cells, macrophages, and CD4 T lymphocytes may play a role in the pathogenesis of asthma (4, 36, 37), increases in these cells have not been consistently reported in the literature (38, 39).

Whether chronic inflammation precedes the development of airway remodeling in asthma is still an open, crucial question. Indeed, the traditional view that airway remodeling is dependent on the prior development of chronic inflammation and would therefore require long periods to become established has recently been questioned (27). Although in our study we found that structural changes were associated with airway eosinophilia, we cannot draw firm conclusions on the temporal relationship between inflammation and remodeling from a cross-sectional study. A properly designed longitudinal study would be required, but it is extremely problematic to perform biopsies in children at different time points.

There are potential criticisms of our study. We acknowledge that a crucial component of airway remodeling, the increase in smooth muscle mass, has not been examined in our report. However, because bronchial biopsies sample only a small portion of the bronchial wall, analysis of smooth muscle is not always possible; this is particularly true in children because biopsies are usually smaller than those from adults. Another potential weakness of this study is that all children underwent bronchoscopy for specific clinical indications other than asthma (mainly recurrent pneumonia, chronic cough, and stridor) (20, 21), and the presence of these pathologic conditions could have influenced the results. However, because these conditions were equally distributed in the three groups of children examined, we are confident that our observation of the presence of airway remodeling in children with asthma is valid. Moreover, biopsies from children undergoing bronchoscopy for clinical indications other than asthma are the only specimens allowing a direct examination of airway pathology in children with mild asthma, which would be otherwise impossible for ethical reasons (40).

Another limitation of our report is the low power of the study because of the small number of subjects in each group. Moreover, we should acknowledge that, because our population included very young children, we performed only one biopsy per child, thus introducing a possible sample bias. However, a low number of subjects and a low number of biopsies per subject are common limitations in biopsy studies, especially in children, in whom invasive maneuvers are more problematic. Finally, we should recognize that there is a large overlap among individual data of the groups, a phenomenon that is quite common in biopsy studies given the high variability in morphometric measurements observed in patients with asthma. Nevertheless, we think that the significant differences we observed are valid even if we cannot exclude the possibility of type 2 statistical errors (i.e., inability to detect small differences). Despite all of these limitations, we believe that studies on bronchial biopsies provide a unique opportunity to investigate airway inflammation and remodeling in childhood asthma.

In conclusion, epithelial damage and reticular basement membrane thickening, which are pathologic features characteristic of adult asthma, are present even in young children with mild/moderate disease. Of interest, other changes, such as airway eosinophilia and angiogenesis, were observed not only in children with asthma but also in atopic children without asthma. These observations suggest that inflammatory and structural changes occur early in the natural history of asthma, but also emphasize the concept that some of these pathologic lesions may be associated with atopy even in the absence of asthmatic symptoms.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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