

Airway Inflammation in Childhood Asthma

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Airway pathology has been extensively investigated in adulthood asthma, whereas only few studies examined bronchial biopsies in childhood asthma. To evaluate the airway pathology in children with asthma, we analyzed bronchial biopsies obtained from 23 children undergoing bronchoscopy for clinical indications other than asthma. Nine had mild/moderate asthma. Six had atopy without asthma, and eight had no atopy or asthma. We measured basement membrane thickness and quantified the number of eosinophils, mast cells, neutrophils, macrophages, T lymphocytes, and positive cells for transforming growth factor- β 1 (TGF- β 1) and its receptors I and II (TGF β -RI and TGF β -RII) in subepithelium. Children with asthma had an increase in basement membrane thickness and in the number of eosinophils compared with control subjects, but not compared with children with atopy. They also had a decreased expression of TGF β -RII compared with both those with atopy and control subjects. In children with asthma, the number of eosinophils correlated negatively with TGF β -RII and positively with symptom duration. In conclusion, airway eosinophilia and basement membrane thickening, which are the pathologic features that are characteristic of adulthood asthma, are already present in children with mild asthma, and even in children with atopy without asthma. Moreover, in children with asthma but not in children with atopy without asthma, there is a downregulation of TGF β -RII.

Keywords: inflammation; remodeling; pediatric asthma; cytokines

Airway inflammation and remodeling play an important role in the pathophysiology of asthma. Although the airway pathology has been extensively investigated in adulthood asthma, only a few studies examined bronchial biopsies obtained from children with asthma (1). Investigating the airway pathology in childhood asthma may be of interest to clarify whether the pathologic features seen in adults begin early in the course of the disease and whether remodeling occurs in parallel with inflammation or sequential to it. Previous studies (2, 3) have described the presence of both inflammation and thickening of the basement membrane in children with asthma. However, in those studies, only a qualitative analysis was performed, and a control group of children without asthma was not included. More recently, Payne and coworkers (4), in a quantitative well-conducted study, demonstrated that children with difficult asthma had a thickened basement membrane as compared with pediatric control subjects and that this thickening was similar to that seen in adulthood asthma. However, as stated by the authors, the children examined in that study are not typical of the majority of children with

asthma but represent those with the most severe disease, with persistent symptoms despite maximal steroid therapy. Furthermore, that study did not include a control group of children with atopy without asthma. Therefore, it is yet to be elucidated whether inflammation and remodeling can be already observed in children when the disease is mild or even when only atopy is present.

There is accumulating evidence to suggest that transforming growth factor- β 1 (TGF- β 1) may be involved in orchestrating both inflammatory and remodeling processes in asthma. TGF- β 1 is a pleiotropic cytokine that through binding to its type I and type II receptors (TGF β -RI and TGF β -RII) can exert a number of biological effects, including profibrotic and antiinflammatory activities (5, 6). Although some studies in human asthma have shown an increased expression of TGF- β 1 (7–9), underlining its profibrotic role, other studies have demonstrated an antiinflammatory activity of this cytokine (10, 11). It could be of interest to investigate the expression of TGF- β 1 and its receptors in childhood asthma, when the onset of inflammation and remodeling processes is probably occurring.

As far as we know, no study has addressed the issue to quantify the airway inflammatory and structural changes in children with mild/moderate asthma and to compare them with the appropriate pediatric control subjects, in particular with children with atopy without asthma. We therefore decided to measure the inflammatory cells and the basement membrane thickness in bronchial biopsies of children with mild/moderate asthma, of children with atopy but without asthma, and of children with no atopy or asthma. Moreover, to clarify the role of TGF- β 1 in airway inflammation and remodeling, we examined the expression of TGF- β 1, TGF β -RI, and TGF β -RII in the three groups of children. Some of the results of these studies have been previously reported in the form of an abstract at the 2003 Meeting of the American Thoracic Society (Seattle, WA) (12).

METHODS

Subjects

We recruited to the study 23 children who had undergone fiberoptic bronchoscopy for appropriate clinical indications other than asthma (13, 14). The study population included the following three groups: nine children with asthma (age of 4–12 years), six children with atopy without asthma (age of 3–13 years), and eight control children without asthma or atopy (age of 4–12 years). Children with asthma underwent bronchoscopy for persistent atelectasis ($n = 3$) or for recurrent pneumonia ($n = 6$). Children with atopy without asthma underwent bronchoscopy for stridor ($n = 1$), persistent atelectasis ($n = 1$), or recurrent pneumonia ($n = 4$). Children who were control subjects underwent bronchoscopy for stridor ($n = 2$), persistent atelectasis ($n = 1$), recurrent pneumonia ($n = 3$), or chronic cough ($n = 2$).

Asthma was diagnosed when the child had episodic cough, breathlessness, and a wheeze responsive to bronchodilators (15). The presence of atopy was defined by an increase in total (paper-radio-immunosorbent-test) or specific (radio-allergo-sorbent-test) IgE. All children

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of the three groups underwent paper-radio-immuno-sorbent-test, radio-allergo-sorbent-test, and routine blood tests, whereas spirometry was performed only in children who were able to cooperate with the test. FEV₁ was measured using a 10-L bell spirometer (Biomedin, Padua, Italy), and the best of three maneuvers was expressed as a percentage of predicted values (16).

Performance of endobronchial biopsy for studying airway pathology was approved by local ethics committees. Informed consent was obtained from the children's parents. The study was performed according to the Declaration of Helsinki. Bronchoscopy was performed as previously described (17), except that the fiberoptic bronchoscope was inserted orally using a mouth Olympus MA-651 (K). One bronchial biopsy specimen was taken from the main carina using a bronchial forceps (Olympus FB 15 C-1) inserted through the service channel of the bronchoscope.

Sample Processing and Analysis

Biopsies were processed as previously described (18). Reticular basement membrane thickness was assessed on sections stained with hematoxylin and eosin by making measurements at 50- μ m intervals along all the basement membrane, using a computer-aided image analysis (Casti Imaging, Venice, Italy). The infiltration of eosinophils, neutrophils, mast cells, macrophages, and CD4 T lymphocytes was assessed in the subepithelium by immunohistochemistry as previously described (19). The expression of TGF- β 1, TGF- β -RI, and TGF- β -RII was assessed in the subepithelium using immunohistochemical methods. Briefly, all biopsy sections were subjected to antigen retrieval by heating in a microwave oven on high power for 8 minutes in 0.01 mol/L citrate buffer (pH 6.0) and then incubated with a mouse monoclonal antibody anti-TGF- β 1 (150 μ g/ml, dilution 1:20; Genzyme Diagnostics, Cambridge, MA) with a polyclonal antibody anti-TGF- β 1 receptor type I (200 μ g/ml, dilution 1:200; Santa Cruz Biotechnology Inc., Santa Cruz, CA) or with a polyclonal antibody anti-TGF- β -RII (200 μ g/ml, dilution 1:200; Santa Cruz Biotechnology Inc.). Before incubation with primary antibody, the sections were treated with a biotin blocking kit (Vector Laboratories, Peterborough, UK) to inhibit endogenous biotin. The detection system was performed using the Vectastain ABC kit (Vector Laboratories) with 3-amino-9-ethylcarbazole as the chromogenic substrate. Sections were counterstained with Mayer's hematoxylin. The surface epithelial layer was not included in the count because of the frequent erosion or loss by technical misprocessing.

To avoid observer bias, the cases were coded, and the measurements were made without knowledge of clinical data. Differences between groups were analyzed using the analysis of variance for clinical data and the Kruskal-Wallis test for histologic data. The Mann-Whitney U test was performed after Kruskal-Wallis test when appropriate. Correlation coefficients were calculated using Spearman's rank method. Probability values of 0.05 or less were accepted as significant. Group data were expressed as means and SEM or as medians and range when appropriate.

RESULTS

Clinical Findings

The characteristic of the children studied are shown in Table 1. Spirometry was successfully performed in six of nine children

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

Characteristics	Children with Asthma	Children with Atopy	Control Children
Number, male/female	5/4	3/3	3/5
Age, yr	8 $\pm$ 1	7 $\pm$ 1	7 $\pm$ 1
Age range, yr	4–12	4–12	3–13
Atopy	6/9	6/6	0/8
Duration of asthma, yr	5.7 $\pm$ 1.2	—	—
FEV ₁ , % predicted	79 $\pm$ 5*	90 $\pm$ 2	102 $\pm$ 4

Values are expressed as absolute numbers or as means $\pm$ SEM.

* $p < 0.01$ as compared with control children.

with asthma, four of six children with atopy, and five of eight control subjects. Children with asthma had a FEV₁ value (percentage of predicted) that was significantly lower than control children ($p = 0.008$). The three groups of children were similar with regard to age and sex. Six out of nine children with asthma were atopic. The bronchoscopy procedure was well tolerated by all children.

Seven of nine children with asthma had mild asthma and were treated with only inhaled salbutamol when needed. The remaining two children had moderate asthma and were treated regularly with combined salmeterol/fluticasone (50/100 twice a day in one child and 50/250 twice a day in the other child). All children with recurrent pneumonia ($n = 13$) were in treatment with antibiotics.

To confirm further the absence of asthma in children with atopy without asthma, we performed exercise challenge in those children who were able to cooperate with the test (three out of six). None of them had a fall in FEV₁ that was 15% or more after exercise.

Biopsy Findings

Quantification of inflammatory cells was satisfactory in all children except in one of the group with asthma and in one of the control group in whom we could quantify only eosinophils and basement membrane thickness because of the limited amount of biopsy tissue. For the same reason, we were not able to quantify TGF- β 1 and its receptors in one child in the group with atopy.

Children with asthma had an increased reticular basement membrane thickness as compared with children who were control subjects (6.0, 4.5–9.5 vs. 4.2, 3.3–4.9; $p = 0.001$) (Figures 1 and 2). Even if to a lesser extent, also children with atopy showed an increased basement membrane thickness as compared with children who were control subjects (4.9, 4.2–6.6 vs. 4.2, 3.3–4.9; $p = 0.052$) (Figure 1). In addition, both children with atopy without asthma and children with asthma had an increased number of eosinophils in the subepithelium as compared with children who were control subjects ($p = 0.033$ and $p = 0.038$, respectively) (Figures 3 and 4 and Table 2). No significant difference was observed between children with atopy and with asthma in the reticular basement membrane thickness or in the eosinophilic infiltration. Of the two children taking inhaled steroids, one had a value of tissue eosinophils in the range of control subjects (48

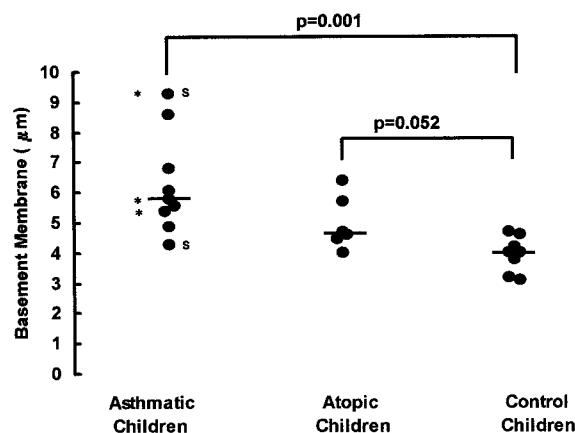


Figure 1. Individual values for reticular basement membrane thickness in bronchial biopsies of children with asthma, children with atopy, and children who were control subjects. Horizontal bars represent median values. The asterisk indicates children with asthma who were not atopic; S indicates children with asthma who were treated with inhaled steroids.

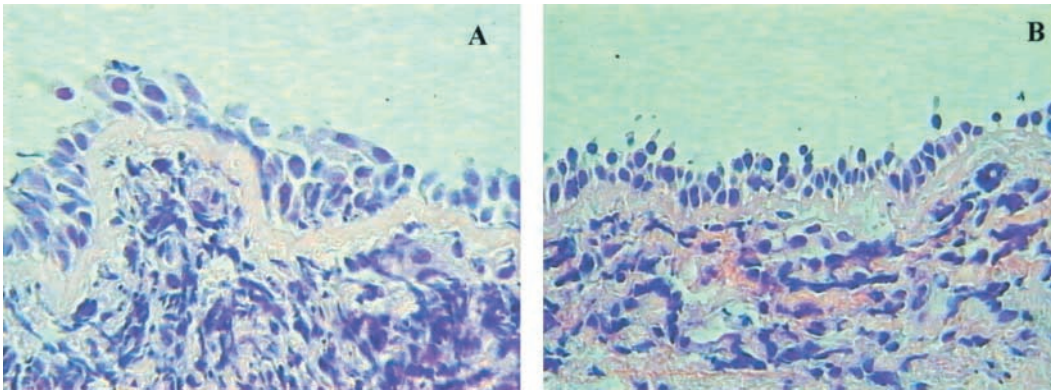


Figure 2. Microphotograph showing bronchial biopsies from a child with asthma (A) and a control child (B), demonstrating an increased basement membrane thickness in the child with asthma. The tissue sections are stained with hematoxylin and eosin. Original magnification, $\times 630$.

cells/mm²), whereas the other had a prominent tissue eosinophilia (338 cells/mm²). No significant differences were observed among the three groups of subjects in the number of neutrophils, mast cells, CD4⁺ T lymphocytes, and macrophages (Table 2). The expression of TGF- β 1 and TGF- β RI was not significantly different in the three groups of children examined (Table 2). However, children with asthma had a decreased expression of TGF- β RII in the subepithelium as compared with both children with atopy and children who were control subjects ($p = 0.004$ and $p = 0.047$, respectively; Figure 5).

When only the group of children with asthma was considered, the number of eosinophils in the airway wall showed a negative correlation with TGF- β RII expression ($p = 0.035$, $r = -0.86$) and a positive correlation with the duration of asthma symptoms ($p = 0.020$, $r = 0.79$). No other significant correlations were observed between cellular counts and functional data or between reticular basement membrane thickness and cellular counts or functional data.

DISCUSSION

This study shows that the pathologic features characteristic of adulthood asthma, that is, airway eosinophilia and basement membrane thickening, are already present in children with mild/

moderate asthma. Moreover, in children with asthma, these pathologic features are paralleled by a decreased expression of TGF- β RII. Children with atopy without asthma also exhibit an increase in number of eosinophils and, even if to a lesser extent, in basement membrane thickness.

By showing that basement membrane thickening is already present in childhood asthma, this study confirms the qualitative observations of previous reports (2, 3). The only study that provided quantitative measurements of basement membrane thickness in childhood asthma has been performed in children with difficult asthma, that is, in children with persistent symptoms despite maximal conventional therapy (4). Moreover, a group of children with atopy without asthma, which, according to the authors, would be the most appropriate control group, was not included in that report. Therefore, to the best of our knowledge, this is the first study to provide a quantification of basement membrane thickening in children with mild/moderate asthma and to compare the results with those of two appropriate pediatric control groups, that is, a group of children with atopy but without asthma and a group of children with no atopy or asthma.

Our observation of an increased number of eosinophils in bronchial biopsies of children with asthma may appear to be in disagreement with previous studies (3, 20). Cokugras and coworkers (3) showed that eosinophilic inflammation was present only in 1 of 10 children with moderate asthma, and Payne and coworkers (20) found that the number of eosinophils in children with difficult asthma was not different from that of children without asthma. However, children examined in those studies had been treated with high doses of inhaled (3) or oral steroids (20), which may have reduced the number of eosinophils. In our study, only two children with asthma were treated with inhaled steroids; thus in the majority of children, we obtained objective measurements of inflammatory cells without the influence of antiinflammatory drugs. The eosinophilic inflammation observed in our study is in keeping with the pioneer observation of Cutz and coworkers, who described a prominent eosinophilia in two endobronchial biopsies and two autopsy samples of children with asthma (2). Moreover, previous studies performed in bronchoalveolar lavage and induced sputum of children with asthma reported an increased number of eosinophils, which was related to the degree of bronchial hyperresponsiveness (21, 22). More recently, analysis of exhaled breath condensate confirmed the presence of an airway inflammatory process in childhood asthma (15, 23).

In this report, airway eosinophilia and, even if to a lesser extent, also basement membrane thickening were already present in

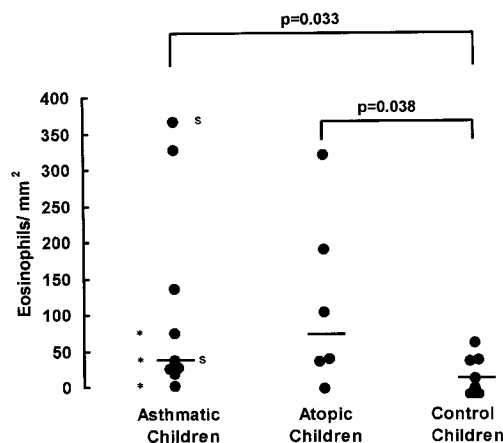


Figure 3. Individual counts for eosinophils in bronchial biopsies of children with asthma, children with atopy, and control children. The results are expressed as number of cells per mm² of tissue examined. Horizontal bars represent median values. The asterisk indicates children with asthma who were not atopic; S indicates children with asthma who were treated with inhaled steroids.

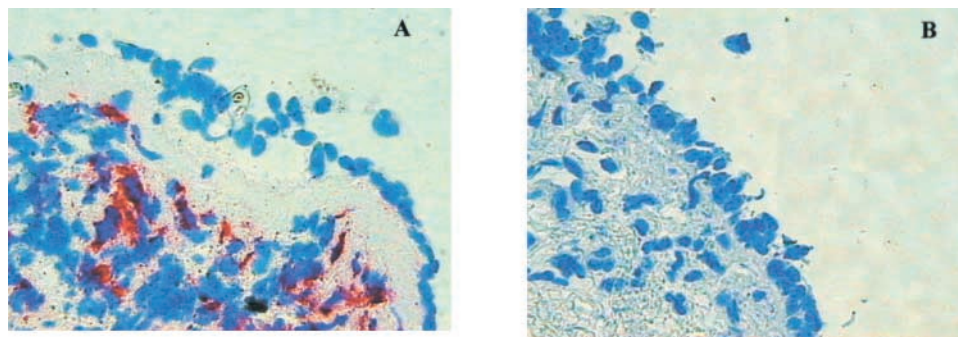


Figure 4. Microphotograph showing bronchial biopsies from a child with asthma (A) and a control child (B), demonstrating an increased number of eosinophils infiltrating the subepithelium in the child with asthma. Immunostaining with monoclonal antibody anti-EG-2 (positive cells are stained in red). Original magnification, $\times 630$.

children with atopy without asthma. The relationship between these pathologic changes, atopy, and asthma symptoms is difficult to establish, especially in children. Indeed, early allergic sensitization seems to play an important role in the development of persistent asthma in the first years of life (24). It can be hypothesized that airway inflammation and remodeling are early lesions that may occur even before the establishment of the disordered lung function characteristic of the disease. On the other hand, as not all children with atopy will eventually develop asthma (25, 26), it is also plausible that these pathologic lesions are not directly related to functional abnormalities. This hypothesis is consistent with the findings of previous studies that reported that basement membrane thickening and airway eosinophilia are present in adults with atopy without asthma (27–30).

One generally accepted hypothesis is that in asthma, airway remodeling is dependent on the prior development of chronic inflammation. Our observation of the presence of both thickening of basement membrane and airway eosinophilia in children with mild asthma suggests that remodeling processes begin early in the course of the disease and most likely occur in parallel with the establishment of the chronic inflammation rather than sequential to it.

To assess the role of TGF- β 1 in modulating the pathways leading to airway remodeling and inflammation, we examined the expression of this cytokine and its receptors in children with asthma. TGF- β 1 is a pleiotropic cytokine that can exert both profibrotic and antiinflammatory activities. The increased expression of TGF- β 1 observed in adults with asthma (7–9) has been traditionally related to tissue repairing processes occurring in the airways damaged by inflammatory cells. It has been hypothesized that the persistent activity of TGF- β 1, induced by chronic inflammation, might have detrimental consequences such as subepithelial fibrosis and airway remodeling (5). How-

ever, other studies have demonstrated that this cytokine may have antiinflammatory activities as well (10, 11), probably through the induction of apoptosis of inflammatory cells, particularly eosinophils (31). In our study, the expression of TGF- β 1 was similar in the three groups of children examined, whereas the expression of TGF- β -RII was decreased in children with asthma as compared with both children with atopy and children who were control subjects. These findings suggest that TGF- β 1 signaling may be downregulated in childhood asthma and therefore may be unable to exert its antiinflammatory activity. This hypothesis is supported by the negative correlation observed between the number of TGF- β -RII⁺ cells and the number of eosinophils in children with asthma. Conversely, neither TGF- β 1 nor its receptors were correlated with basement membrane thickness, suggesting that the profibrotic activity of this cytokine does not contribute to airway remodeling in childhood asthma.

Interestingly, in our population of children, the downregulation of TGF- β -RII expression was the only pathologic feature that was able to differentiate children with asthma from children with atopy without asthma, despite the presence of a similar eosinophilia and a similar basement membrane thickening in the two groups of children. These findings suggest a possible role for the impaired TGF- β signaling in the clinical manifestations of asthma in childhood.

A potential weakness of this study is that all children underwent bronchoscopy for specific clinical indications other than asthma (recurrent pneumonia, persistent atelectasis, chronic cough, and stridor) (13, 14), and the presence of these pathologic conditions, particularly pneumonia, could have influenced the results. However, because these conditions were equally distributed in children with asthma, children with atopy, and children who were control subjects, we feel rather confident that our observation of the presence of pathologic lesions in children

TABLE 2. CELLULAR COUNTS IN THE SUBEPITHELIUM

	Children with Asthma	Children with Atopy	Control Children
Eosinophils	48 (13–376)*	81 (8–330)*	15 (0–72)
Neutrophils	87 (16–244)	98 (19–225)	90 (38–268)
Mast cells	23 (0–132)	93 (0–213)	56 (0–157)
CD4 T-lymphocytes	89 (42–535)	259 (97–357)	213 (11–316)
Macrophages	175 (56–344)	138 (68–225)	137 (11–244)
TGF- β 1 ⁺ cells	182 (66–354)	172 (78–372)	87 (9–470)
TGF- β -RI ⁺ cells	623 (291–1167)	550 (308–1381)	952 (196–1,092)
TGF- β -RII ⁺ cells	179 (47–332)*†	543 (391–676)	479 (71–948)

Definition of abbreviations: TGF- β 1 = transforming growth factor- β 1; TGF- β -RI = TGF- β receptor I; TGF- β -RII = TGF- β receptor II.

Values are expressed as cells/mm².

Values are expressed as median (range).

* $p < 0.05$ as compared with control children.

† $p < 0.05$ as compared with children with atopy.

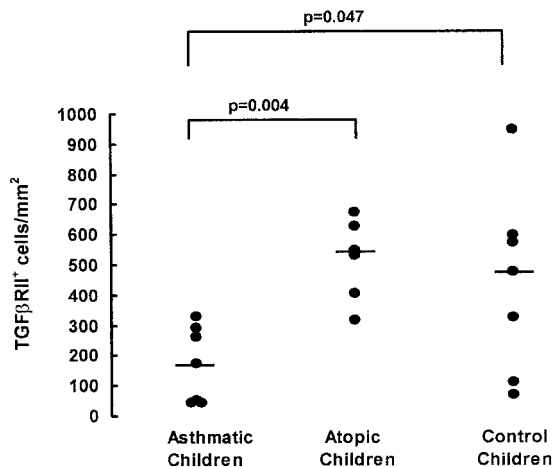


Figure 5. Individual counts for TGF- β RII⁺ cells in bronchial biopsies of children with asthma, children with atopy, and control children. The results are expressed as number of cells/mm² of tissue examined. Horizontal bars represent median values.

with atopy and asthma but not in normal control subjects is valid. Moreover, biopsies from children undergoing bronchoscopy for clinical indications other than asthma are the only specimens allowing direct examination of airway pathology in children with mild asthma, which would be otherwise impossible for ethical reasons (32).

Another limitation of our report is the low power of the study because of the small number of subjects in each group. However, this is a common problem in biopsy studies, especially in children, in whom invasive maneuvers are more problematic. Even if only multicenter studies collecting high numbers of biopsies could overcome these difficulties, we believe that our study provides preliminary data that could prove useful for the design of future research in this field.

Finally, we should acknowledge that because our population included very young children, we performed only one biopsy per child, thus introducing a possible sample error. 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, this study shows that airway eosinophilia and basement membrane thickening, which are the pathologic features characteristic of adulthood asthma, are already present in children with mild asthma and even in children with atopy without asthma. Moreover, we found that in children with asthma, but not in children with atopy without asthma, there is a down-regulation of TGF- β RII.

Conflict of Interest Statement: A.B. has no declared conflict of interest; G.T. has no declared conflict of interest; S.B. has no declared conflict of interest; E.B. has no declared conflict of interest; F.C. has no declared conflict of interest; M.T. has no declared conflict of interest; R.Z. has no declared conflict of interest; B.B. has no declared conflict of interest; P.M. has no declared conflict of interest; L.M.F. has been reimbursed by industries for attending several conferences, has participated as a speaker in scientific meetings or courses organized and financed by various pharmaceutical companies (AstraZeneca, Boehringer Ingelheim, Byk Gulden-Altana, Ciesi Farmaceutici, GlaxoSmithKline, Menarini, Merck, Sharpe & Dohme, Schering Plough), served as a consultant to AstraZeneca, Boehringer Ingelheim, Byk Gulden-Altana, Ciesi Farmaceutici, GlaxoSmithKline, Miat, Roche, Schering Plough and received honoraria for speaking at sponsored conferences and received research grants for participating in multi-center clinical trials and his institution has received educational and research grants from many pharmaceutical companies; M.S. has been reimbursed by various pharmaceutical companies (Glaxo-SmithKline, AstraZeneca and Merck, Sharpe & Dohme) for attending several conferences and has participated as a speaker in scientific meetings or courses organized and financed by GlaxoSmithKline, AstraZeneca and Merck, Sharpe & Dohme.

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