

Original article

Airway remodeling is correlated with obstruction in children with severe asthma

Background: Severe asthma may involve an irreversible obstructive pattern, and structural changes in bronchial airways are believed to play a key role in this context. The aim of the present study was to compare airway remodeling in severe asthmatic children with or without obstructive pattern.

Methods: Two groups of children with severe asthma and persistent symptoms, 5–14 years old were included, 15 with persistent obstructive pattern (group O) and 10 without obstructive pattern (group N). Persistent obstructive pattern was defined as a forced expiratory volume in 1 s (FEV₁) less than 80% of the predicted value after a course of systemic corticosteroids and no significant improvement after bronchodilator. We examined bronchial biopsies by pathological and immunochemical methods and quantified airway smooth muscle (ASM) and mucus gland areas, reticular basement membrane (RBM) thickening, distance between ASM and RBM, muscle light chain kinase (MLCK) expression and number of vessels (CD31 expression).

Results: Surface area of ASM ($P = 0.009$), MLCK expression ($P = 0.03$) and number of vessels ($P = 0.0008$) were increased in group O compared with group N. Distance of RBM–ASM was shorter in group O ($P = 0.007$). FEV₁ negatively correlated with ASM area ($r = -0.6$; $P = 0.002$), MLCK expression ($r = -0.45$; $P = 0.02$) and CD31 expression ($r = -0.7$; $P = 0.0003$), and positively correlated with the distance of RBM–ASM ($r = 0.5$; $P = 0.007$).

Conclusions: Structural abnormalities of airway remodeling are present in children with severe asthma. Only an increase in surface area of ASM and the density of the vascular network are more pronounced in children with persistent obstructive pattern, while RBM thickening is similar. These results are concordant with longitudinal studies which emphasize the precocity of bronchial obstruction.

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Severe asthma in children includes different clinical, functional and pathophysiological phenotypes (1, 2). Among children with persistent symptoms, some patients have a normal forced expiratory volume in 1 s (FEV₁), while others show persistent, irreversible bronchial obstruction despite optimal treatment. In adult asthma, clinical severity has often been linked to an inflammatory and remodeling reaction (3–9). Benayoun et al. (10) showed that increased areas of mucus glands and smooth muscle, a higher number of fibroblasts and an increase in collagen deposition may differentiate severe from mild asthma. The different components of airway remodeling have been poorly investigated in childhood asthma and were limited to reticular basement membrane (RBM) thickness and, more recently, angiogenesis (11). We previously showed, in children with severe asthma and nonreversible bronchial obstruction despite optimal treatment, that the persistence of eosinophils and neutrophils

in the bronchial intraepithelial area was associated with persistent symptoms (2). As this pattern of persistent obstruction is not simply related to persistent inflammation, another possible explanation might be the presence of altered structures of the bronchial walls. In order to determine the potential role of airway remodeling on the profile of pulmonary function tests, we evaluated airway structural changes such as RBM, airway smooth muscle (ASM), mucus glands and vessels in two groups of children with uncontrolled severe asthma, with or without persistent obstructive pattern.

Patients and methods

Patients

Twenty-five children (5–14 years old) with severe asthma were studied. Some of them had already been included in a previous

study (2). These children attended the clinic every 2–3 months for at least 1 year. Symptoms (Juniper control test), bronchodilator use and exacerbations were recorded and lung function tests with post- β_2 -agonist FEV₁ were performed at each visit. Asthma was diagnosed according to the Global Initiative for Asthma (GINA) (12). All children had a history of recurrent episodes of airway obstruction and reversibility of bronchial obstruction or nonspecific bronchial hyper-responsiveness. All children had severe asthma, as defined by the American Thoracic Society (ATS) workshop recently reviewed by S. Wenzel (1). Severe asthma was defined as the presence of one major criterion, i.e. the need for high-dose inhaled corticosteroids, and at least two of the following five minor criteria: (i) the need for a daily long-acting β_2 -agonist or leukotriene antagonist in addition to inhaled corticosteroids; (ii) asthma symptoms requiring daily or near-daily use of a short-acting β_2 -agonist; (iii) persistent airway obstruction (FEV₁ < 80% predicted); (iv) one or more emergency care visits for asthma per year; and (v) three or more oral steroid 'bursts' per year.

Cystic fibrosis, primary ciliary dyskinesia, bronchopulmonary dysplasia, postinfectious pulmonary sequelae, immune deficiencies and vocal cord dysfunctions were excluded. Atopic status was determined by standard skin prick tests and by measuring total serum immunoglobulin E (IgE) levels and levels of IgE specific for house dust mite, cat, grass pollen and cockroaches. Skin prick tests were considered positive if the wheal was 3 mm larger than the negative control to one or more allergens, or if specific IgE levels were at least 3.5 kU/l.

We assigned the children to two groups based on pulmonary function test data. Group N ($n = 10$): children with persistent symptoms defined as the use of more than three bronchodilator rescue treatments per week and/or exacerbations defined as asthma attacks requiring systemic corticosteroids (CS) and/or hospitalization (more than three exacerbations in the past year) but normal FEV₁. Group O ($n = 15$): children with persistent symptoms (as described before) and persistent obstructive pattern (FEV₁ less than 80% of the predicted value). They were submitted to a course of CS (2 mg/kg/day of prednisolone for 14 days), defined as an increase in FEV₁ of less than 15% (and nonsignificant bronchodilator response), that was negative in all cases. Bronchoscopy was performed 4–6 weeks after the course of CS, during a period free of exacerbation and/or acute respiratory infection.

Ethical approval

The study, in particular because of the need for performing endobronchial biopsies to study airway inflammation and remodeling (13), was approved by the ethics committee (CCPPRB) of Necker Enfants-Malades University Hospital, Paris. Written informed consent was obtained from both parents.

Spirometry

Spirometry and flow volume curves were performed according to ATS guidelines (Gould 9000; Sensormedics, Dayton, OH, USA) (14). The pulmonary function test results were obtained after 14 days of prednisolone and post- β_2 -agonist.

Bronchoscopy with endobronchial biopsies

Flexible bronchoscopy procedures were performed under deep sedation (2) with a 4.9 mm external diameter bronchoscope (Olympus Winter IBE GMBH, Hamburg, Germany). Four to six

biopsy samples were taken from segmental or subsegmental bronchi of right or left lower lobes, using cupped forceps.

Tissue processing

Biopsy samples were fixed in 10% formaldehyde and embedded in paraffin blocks. Bronchial biopsies with an altered structure (a defect in some part of the biopsy such as the epithelium layer) were not used and at least two biopsies per patient were studied. They were stained with hematoxylin, eosin and May-Grünwald-Giemsa by standard methods. In sections stained with May-Grünwald-Giemsa, the numbers of inflammatory cells were assessed in the epithelium layer and in the submucosa (beneath the epithelial basement membrane). Results are expressed as the number of cells per millimetre length of basement membrane and per square millimetre of submucosal area, respectively. The mucus gland areas were excluded from counting.

For evaluation of airway structural changes, sections of 5 μ m were collected on silane-coated glass slides. Slides were first deparaffinized and then treated for antigen retrieval either with 0.05% Triton solution in phosphate-buffered saline [PBS; for epidermal growth factor (EGF) and transforming growth factor, TGF- β], heating for 15 min at 95°C in citrate buffer [for EGF receptor (EGFR), myosin-light chain kinase (MLCK) and CD31] or incubation in 0.1% trypsin type II in Tris buffer for 10 min (for α -smooth muscle actin, SMA). A hematoxylin-stained section was also prepared. Immunohistochemistry was performed with goat IgG anti-collagen type I, monoclonal antibodies (mAb) anti-EGF and TGF- β (mAb 1032 and 126, respectively; Chemicon, Temecula, CA, USA), mAb anti-SMA (clone asm-1; Cymbus Biotech, Southampton, UK), MLCK (clone K36; Sigma Co, Saint-Quentin Fallavier, France), EGF-R (clone 111.6) and CD31 (clone JC30A; Dako, Trappes, France). Primary antibody binding was detected with the appropriate Vectastain ABC kit (Vector, Burlingame, CA, USA) which included a complex of avidin and biotinylated alkaline phosphatase.

The intensity of collagen type I deposition was quantified as absent (0), weak (1), moderate (2) or high (3). The same evaluation was performed for anti-EGF, anti-EGF-R and anti-TGF- β antibodies. The scale was arbitrarily defined after an exhaustive review of all the specimens and of the negative controls. The number of structures stained with anti-CD31 mAb and anti-MLCK was determined on the area of at least two grids (surface 0.59 mm²) per biopsy and on two biopsies per patient, and reported as the mean number of positive section or cells/mm², respectively.

Biopsies were examined at $\times 500$ magnification. For each biopsy, the granulocyte count was performed on the area of two or more grids (surface 0.59 mm² for subepithelial area; side length 0.4 mm for intraepithelial count). Morphometry was assessed by computer-assisted image analysis performed in at least three noncontiguous areas randomly chosen and expressed in square millimetre (Leica Microsystems, Nussloch, Germany). RBM thickening (in μ m) was measured as the average of 20 measurements performed at regular intervals of 50 μ m (15). Epithelial integrity was determined as the percentage of length of the basement membrane with intact epithelium on the total of both biopsies (10). The percentage of the area of bronchial mucosa occupied by mucus glands and ASM was determined in all the biopsy on hematoxylin-stained and anti-SMA antibody-treated section, respectively. For this, the bundles of ASM or the mucus glands were covered by a line drawn and then the surface was calculated by the program of image analysis. All the ASM bundles are analysed independently of their orientation. The distance between an area stained by SMA and the RBM was reported in micrometres. For these measurements, we did not take

into account the area of bronchial mucosa with shrinkage. At least 10 measurements were assessed for each section at 50 µm regular intervals when possible. The parameters were evaluated by two independent observers blinded to the study code. The results are expressed as the mean of all measurements obtained for each patient. For all the parameters, the coefficient of variation for two sections from the same biopsy was below 5% and between biopsies from the same patient was 8–13%.

Statistical analysis

Results are expressed as median with the 25th and 75th centiles. Differences between groups were assessed using the Mann-Whitney *U* test for continuous variables and exact Fisher's test for qualitative variables. Univariate regression for correlation was performed using Spearman's rank-order method. A *P*-value less than or equal to 0.05 was considered significant.

Results

The characteristics of patients and results are described in Table 1. None of the children was active smoker. Bronchoscopy and endobronchial biopsies were well tolerated. Duration of asthma, treatment and number of exacerbations did not differ between the two groups. Agreement between the two observers was good. The interclass correlation coefficient was 0.997 and the coefficient of variation did not exceed 11%.

Eosinophil and neutrophil counts in the bronchial mucosa

Eosinophil and neutrophil counts in the intraepithelial area and the mucosa did not differ between the two groups. In the intraepithelial area, eosinophil counts for groups O and N were 5 (2.5–9.4) and 2.5 (0–6.2) respectively, and neutrophil counts were 10 (7.5–13.7) and 7.5 (5–8.7). Concerning the bronchial mucosa, eosinophil counts were 16.9 (5.5–16.9) and 8.47 (5.5–

26.7) in groups O and N, respectively, whereas neutrophil counts were 45 (27.9–61.4) and 30.5 (22.8–66), respectively.

Epithelial integrity and RBM thickness

The percentage of epithelial integrity [41% (11.2–70.1) vs 77% (40–90)] and RBM thickness [6.7 µm (5.4–9.6) vs 7.6 µm (6.1–8.7)] were not significantly different between groups O and N.

Expression of collagen type I, EGF, EGF-R and TGF-β

Intensity of staining evaluated with a score between 0 and 3 did not differ between groups O and N for collagen type I [2 (1, 2) and 1 (1–1.5)], EGF [2 (1.75–2.25) and 2 (1–2)], EGF-R [1 (0–2) and 1 (0.5–1)] or TGF-β [1 (1–2) and 1 (1–1.7)].

Distance between RBM and ASM, ASM area and MLCK expression

The distance between RBM and ASM was shorter in group O [10 µm (8.5–30)] than in group N [40 µm (30–64)] (*P* = 0.007) (Fig. 2A). ASM area was significantly higher in group O [24% (17–31.5)] than in group N [15% (11–20)] (*P* = 0.009) (Fig. 2B). The number of cells positive for MLCK was significantly higher in group O [35 (19.5–45)] than in group N [12 (9–16.2)] (*P* = 0.03) (Fig. 2C).

Mucus gland area and vascular structure counts

The mucus gland area did not differ between the two groups [9.5% (2.9–20.7) and 4.8% (2.5–8) for groups O and N, respectively] as illustrated in Fig. 1 with a low magnification. In contrast, the number of vascular structures identified in bronchial mucosa was significantly

Table 1. Clinical characteristics of children with severe asthma

	Group O (<i>n</i> = 15)	Group N (<i>n</i> = 10)	<i>P</i> -value
Sex ratio (M/F)	7/8	7/3	NS
Age (years)*	10.9 (6–14)	8.9 (5–13)	NS
Age at onset (years)	1 (0.9–1.9)	1.5 (0.3–2)	NS
Duration of asthma (years)	9.85 (7.9–11)	8.3 (7.1–10.2)	NS
Acute exacerbation (number per year)	5 (3–6)	7 (3–9)	NS
Duration of inhaled corticosteroid treatment (years)	5 (3.7–7)	4. (3–7.5)	NS
Inhaled corticosteroids (µg/day eq. beclomethasone)	1000 (850–1000)	1000 (800–2000)	NS
FEV ₁ (% predicted)	73 (67.2–75.5)	87 (82–97)	0.0006
FEF 25–75 (% predicted)	47 (33.2–53.7)	67 (58–85)	0.0016
FEF 50 (% predicted)	38 (27.5–61)	80 (60.5–88)	0.014
FEF 25 (% predicted)	35 (28.5–47)	62.5 (48.5–77)	0.005
Total serum immunoglobulin E (IgE; kU/l)	210 (20–690)	230 (124–554)	NS
Positive skin prick test or specific IgE (<i>n</i>)	11	7	NS
Systemic corticosteroid courses per year	3.5 (1–6)	6 (3–8)	0.08

Group O, children with persistent airway obstruction; group N, children without persistent airway obstruction; NS, not significant; FEV, forced expiratory volume. Results are expressed as median (25th–75th centiles) except for age (*) expressed as median (range).

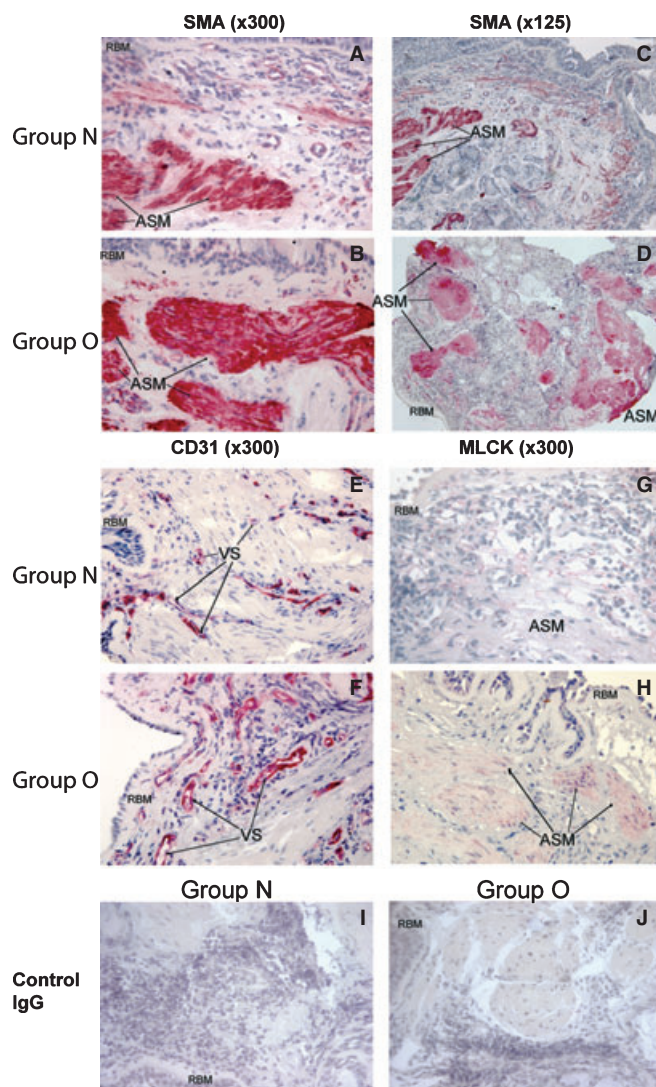


Figure 1. Histopathologic features of airway remodeling in bronchial biopsies from children with severe asthma, associated (group O) or not (group N) with persistent airway obstruction. The four upper photos (A–D) report data obtained with anti- α -smooth muscle actin (SMA) antibody labeling. At high magnification ($\times 300$), there is a shorter distance between reticular basement membrane (RBM) and airway smooth muscle (ASM) in group O than in group N (photos A, B). Moreover, the ASM area was larger in group O than in group N; this is also illustrated at lower magnification in a section obtained from one patient from each group (photos C, D). The four photos in the middle (E–H) report data obtained with anti-CD31 and anti-muscle light chain kinase (MLCK) antibodies. Numerous vessel sections (VS) were observed in group O compared with group N, as shown by CD31 staining (photos E, F). Restrained MLCK staining was observed in the ASM area in group O (as confirmed by SMA staining in adjacent section), whereas it was nearly absent in group N (photos G, H). The two bottom photos (I, J) illustrate the absence of staining obtained with a mouse immunoglobulin (Ig)G1 isotype control in the two groups.

higher in group O [23 (18–32)] than in group N [11 (8–17)] ($P = 0.0008$) (Fig. 2D).

Correlations between duration of asthma, duration of treatment with inhaled corticosteroids, FEV₁ and structural changes

There was no correlation between the duration of asthma and remodeling parameters, nor between duration and doses of anti-inflammatory drugs and remodeling parameters. FEV₁ was positively correlated with the distance between RBM and ASM ($r = 0.5$; $P = 0.007$) and was negatively correlated with ASM area ($r = -0.6$; $P = 0.002$), MLCK expression ($r = -0.45$; $P = 0.02$) and CD31 expression ($r = -0.7$; $P = 0.0003$) (Fig. 3A–D). There were no differences in remodeling parameters between atopic and nonatopic children (data not shown).

Discussion

In the present study, we demonstrate that in a group of children with severe asthma and poorly controlled asthma, patients showing a persistent obstructive pattern (group O) had significantly more structural changes, as assessed by a larger smooth muscle area, increased MLCK expression, a shorter distance between smooth muscle and the RBM, as well as a higher number of vessels into the bronchial mucosa, when compared with children without chronic obstructive pattern (group N). These parameters were correlated with the level of bronchial obstruction, assessed by FEV₁ and FEF 25–75%, but not with the duration of asthma and the dose or duration of inhaled corticosteroids. This is the first study quantifying such a large number of structural changes associated with airway remodeling in children with a history of severe asthma.

Mechanisms and consequences of remodeling remain poorly understood, particularly in asthmatic children. The decline in FEV₁ is accelerated and/or persistent in some children, sometimes despite the use of early and repeated courses of steroids, moreover it may occur early in life (16–18).

In a study evaluating the biopsies of six children with severe asthma, the authors showed thickened subepithelial membrane, goblet cell hyperplasia and elastin deposition, but airway architecture did not predict the clinical response (8). RBM thickening is a major determinant of the presence of remodeling, but did not differ between both groups in the present study. We found no correlation between RBM thickness, epithelial integrity and clinical characteristics or lung function tests, in agreement with other pediatric studies (19–21). Nevertheless, evaluating epithelial integrity is controversial when the biopsy procedure may alter epithelial integrity by itself.

Among factors which may be implicated in structural changes, TGF- β is increased in severe asthma and in status asthmaticus (8, 22, 23). ASM cells release TGF- β

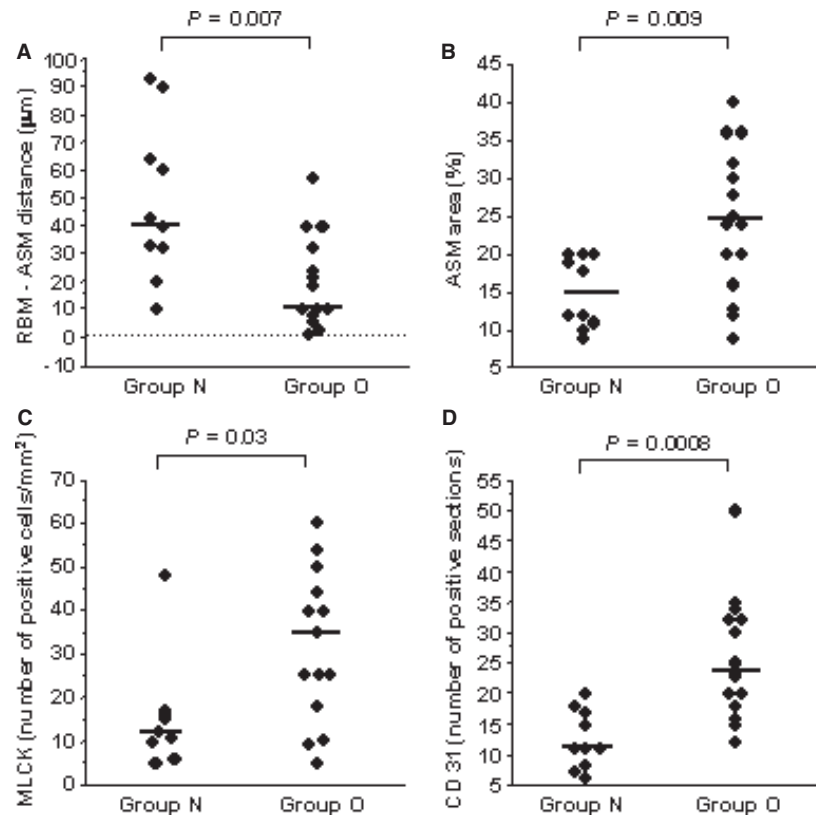


Figure 2. Results obtained in patients of groups O and N for the distance between airway smooth muscle (ASM) and reticular basement membrane (RBM; A), the percentage of ASM area (B), the number of muscle light chain kinase (MLCK)-positive cells (C) and the number of CD31-positive sections (D). The horizontal bars represent the median for each group. Differences between groups were assessed using the Mann-Whitney *U* test.

which induces autocrine and paracrine synthesis of collagen (24). Activated eosinophils are a major source of this cytokine (25). This is one way to link inflammatory processes with structural changes. Results on collagen deposition are conflicting. Expression of type I and III collagens correlated with asthma severity (10). In contrast, collagen type I and III deposition did not predict clinical severity of the disease in other studies (26). We found no difference in collagen and TGF- β expression between both groups. However, our results were limited to proximal airways. More data concerning small airways would be of interest in this population of severe asthmatic children. In addition, the method of quantification is not precise and more data are required before concluding on these parameters.

Overexpression of EGF-R as another marker of epithelial damage was demonstrated in asthmatics when compared with nonasthmatic children (27). However, in our study, we were unable to identify differences between obstructive and nonobstructive patterns concerning the EGF and EGF-R expression. These data suggest that this pathway of cell activation may not play a key role in persistent airway obstruction. However, we cannot exclude the participation of other EGF-R ligands.

Our results emphasize the importance of smooth muscle hyperplasia/hypertrophy and vascular changes in airway remodeling. The surface of the smooth muscle was significantly higher in asthmatic children with a persistent obstructive pattern, and was negatively correlated with FEV₁ and FEF 25–75%. The distance between muscle area and RBM was lower in children with persistent obstruction, and was correlated with FEV₁ and FEF 25–75% values. Hyperplasia and hypertrophy are important processes regulating increased smooth muscle mass in asthma (28). Myofibroblasts display a phenotype intermediate between fibroblasts and smooth muscle cells, express α -SMA and have the ability to secrete matrix proteins and chemokines that prolong eosinophil survival (29). In patients dying of status asthmaticus, the amount of smooth muscle is increased from the large to the small airways (30). The negative correlations between lung function alterations and the SMA area are in agreement with the results of Benayoun in adults (10). Smooth muscles encircle central and peripheral airways and muscular shortening produces constriction and shortening of the airways (9). This could contribute to loss of lung function. However, remodeling process may have protective effects. Increased ASM or ACM could

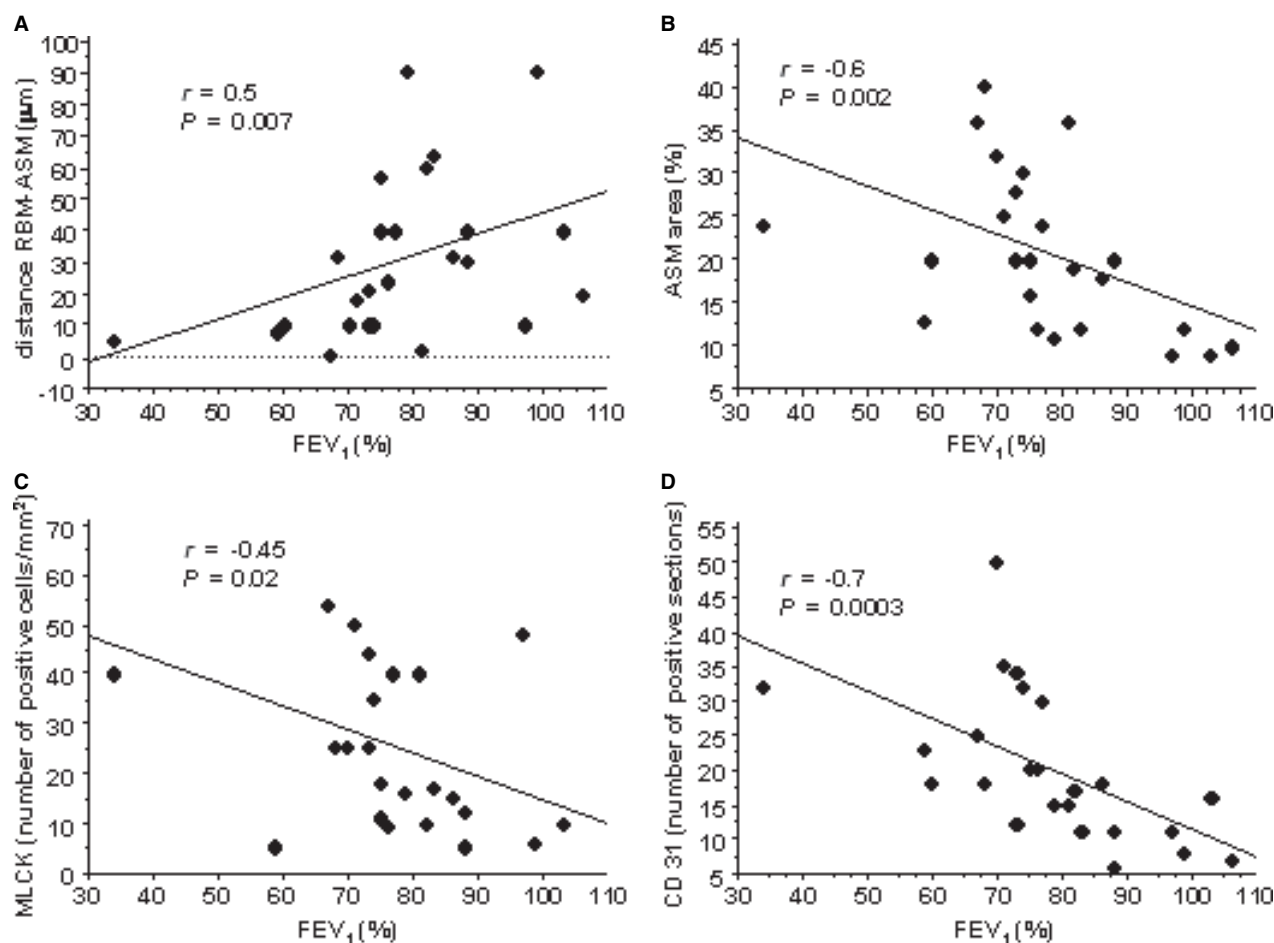


Figure 3. Correlations between forced expiratory volume in 1 s (FEV_1) and distance between airway smooth muscle (ASM) and reticular basement membrane (A), ASM area (B), muscle light chain kinase expression (C) and CD31 expression (D) in children with severe asthma. Univariate regression for correlation was performed using Spearman's rank-order method. P -value less than or equal to 0.05 was considered significant.

minimize shortening by adding the loads against which smooth muscle shortens (30) but the consequence of the findings are debated. One hypothesis is that structural alterations can lead to fixed airway obstruction (31). Previous studies suggested that asthmatics have less distensible airways than nonasthmatic healthy subjects (32, 33). The role of airway smooth muscle on airway distensibility is debated, but may more likely be due to remodeling of the muscle rather than an increase in contractility (34). The increased thickness of ASM leads to excessive airway narrowing during bronchoconstriction, and could increase the load against which airway-distending forces act, making the airways stiffer. This may be amplified by other structural changes. Stiffened airways result in reduced distensibility and lower maximal airway caliber at total lung capacity (35). Brackel et al. (36) showed that the central airways of patients with stable asthma are less compressible during forced expirations compared with healthy controls, concluding that, during broncho-constriction, increased airway stiffness

may confer a protective effect by increasing the load on ASM. This may confer a protective effect in asthma during bronchoconstriction, but potentially detrimental during lung inflation.

MLCK is a protein involved in smooth muscle contraction (37). Smooth muscle MLCK mRNA was found to be higher in asthmatic bronchial smooth muscle cells than in control cells. The relevance of the increased MLCK expression in the present study remains unclear. An increase in kinase message content was an index of the mechanism for increased velocity and capacity of shortening. These changes may account for the increased contractility of bronchial smooth muscle cells and bronchial obstruction (10). The increased MLCK expression in the group with persistent obstructive pattern might be associated with an increased shortening of ASM but remains to be explored.

The mucus gland area did not significantly differ in the two groups of asthmatic children, in contrast with the higher proportion of submucosal gland area in adults

with severe asthma and fatal or nonfatal asthma (38). Hypersecretion was rare in our population of relatively young asthmatic children.

Our study emphasizes the role of vascular changes in airway wall thickening. An increase in vessel number and diameter has been reported in asthmatic patients, and was correlated with the severity of asthma (11, 39, 40). The present study revealed a marked increase in vessel structures, as assessed by CD31 expression in children with chronic obstruction, which was negatively correlated with FEV₁ and FEF 25–75% values. Increased vessel numbers resulting from angiogenesis, vasodilatation and edema contribute to airway wall thickening (41). Furthermore, adhesion of inflammatory cells to the bronchial wall vessels is dependent on adhesion molecules, which are highly expressed on endothelial cells in asthma (42).

Evaluation of inflammation based on bronchial biopsies analysis did not differ between both groups. The discrepancy results for inflammation evaluation in this study compared with a previous study (2), is related to the difference in children included. In the previous study, all children had a persistent obstructive pattern, but one group included pauci-symptomatic children, and the other group symptomatic children. In contrast, in the present study, all children were symptomatic, one had persistent obstruction and another had normal lung function. The link between inflammation and remodeling is debated. Chronic inflammation might initiate tissue injury and repair although remodeling may occur in parallel with inflammation. We have no answer based on our results to definitively conclude in the present study.

Another point must be stressed: the link of remodeling with the duration of asthma and the age of patients. Structural changes in adults with severe asthma are related to the duration of the disease (6, 9). Airway narrowing was more pronounced in older than in younger individuals with fatal asthma (6). In 228 adult asthmatics, after 26 years of follow-up, risk factors for the development of irreversible airway obstruction (FEV₁ < 80% predicted) were analyzed (43). The development of irreversible airway obstruction, diagnosed in 16% of asthmatics, was related to a lower FEV₁ and less reversibility of airway obstruction at initial screening. Limb et al. (44) showed irreversible lung function deficits in 84 young adults with a history of moderate-to-severe asthma in childhood. Irreversible obstruction in adults was associated with a longer duration of asthma, increased childhood metacholine sensitivity and birth prematurity. Although corticosteroid may be implicated in the downregulation of mediator implicated in remodeling such as protease (45, 46), the present study failed to identify a correlation between duration of asthma, duration of treatment and remodeling parameters. This

observation argues for the existence of different phenotypes in severe asthma.

We are aware that this study had some limitations. It included a highly selective subpopulation of children with severe asthma followed at one medical center (Necker University Hospital) and a relatively small number of patients. Data concerning the results of metacholine challenge were not available. It was performed in a small number of children, when others were too severe at any time to evaluate the level of bronchial hyper-responsiveness. Present results need to be confirmed by clinical and functional follow-up of this cohort, and especially of those children with normal pulmonary function tests and a lesser extent of remodeling. For ethical reasons, such a study may be difficult to perform in our institution.

In conclusion, the main determinants of airway remodeling are significantly more important in a subgroup of severe asthmatic children with persistent obstructive pattern, probably defining a subgroup in asthmatic children. These specific structural changes (smooth muscle and vessels) are increased in children with reduced lung function, and although clinically the two groups are similar, the difference in lung function seems to relate to a difference in degree of vascular and smooth muscle airway remodeling, but not other features such as RBM thickness. The hypothesis that airway remodeling is a response to chronic bronchial inflammation remains uncertain, especially in children with severe asthma and chronic irreversible obstruction. We failed to identify a difference in eosinophil and neutrophil infiltrates in bronchial biopsies between the two groups. Similarly, in adult asthma, remodeling was not associated with a high level of granulocyte infiltrate (10). One previous study reported an inverse relationship between CD4⁺ T cells and FEV₁ in children with severe asthma (47). The involvement of T lymphocytes in lung remodeling in our groups of patients remains to be determined. The absence of a difference in asthma duration between obstructive and nonobstructive asthmatic children suggests that structural changes occur early in the natural history of asthma (48). Longitudinal studies emphasize the precocity and persistence of both clinical severity and bronchial obstruction from childhood to adulthood (49, 50). The fact that structural changes, mainly in smooth muscles and vessels, are correlated with lung function alterations and are poorly reversible with inhaled corticosteroids represents a major therapeutic challenge (51, 52).

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