

Airway Remodeling in Preschool Children with Severe Recurrent Wheeze

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Abstract

Rationale: Airway wall structure in preschoolers with severe recurrent wheeze is poorly described.

Objectives: To describe airway wall structure and inflammation in preschoolers with severe recurrent wheeze.

Methods: Flexible bronchoscopy was performed in two groups of preschoolers with severe recurrent wheeze: group 1, less than or equal to 36 months ($n = 20$); group 2, 36–59 months ($n = 29$). We assessed airway inflammation, reticular basement membrane (RBM) thickness, airway smooth muscle (ASM), mucus gland area, vascularity, and epithelial integrity. Comparisons were then made with biopsies from 21 previously described schoolchildren with severe asthma (group 3, 5–11.2 yr).

Measurements and Main Results: RBM thickness was lower in group 1 than in group 2 (3.3 vs. 3.9 μm ; $P = 0.02$), was correlated with age ($P < 0.01$; $\rho = 0.62$), and was higher in

schoolchildren than in preschoolers (6.8 vs. 3.8 μm ; $P < 0.01$).

ASM area was lower in preschoolers than in schoolchildren (9.8% vs. 16.5% ; $P < 0.01$). Vascularity was higher in group 1 than in group 2 ($P = 0.02$) and group 3 ($P < 0.05$). Mucus gland area was higher in preschoolers than in schoolchildren (16.4% vs. 4.6% ; $P < 0.01$). Inflammatory cell counts in biopsies were not correlated with airway wall structure. ASM area was higher in preschoolers with atopy than without atopy (13.1% vs. 7.7% ; $P = 0.01$). Airway morphometrics and inflammation were similar in viral and multiple-trigger wheezers.

Conclusions: In preschoolers with severe recurrent wheeze, markers of remodeling and inflammation are unrelated, and atopy is associated with ASM. In the absence of control subjects, we cannot determine whether differences observed in RBM thickness and vascularity result from disease or normal age-related development.

Keywords: preschool asthma; severe recurrent wheeze; bronchial biopsy; airway remodeling; children

Structural changes to the airway wall, referred to as remodeling, are a characteristic feature of mild to severe forms of adult asthma (1). Markers of airway remodeling are epithelial injury, thickening

of the reticular basement membrane (RBM), goblet cells and airway smooth muscle (ASM) hypertrophy and hyperplasia, and angiogenesis (2). Most of these characteristics are observed early in

the course of asthma, in school-aged children with asthma (3–9), but it remains unclear when precisely remodeling begins. This is a crucial issue because although prenatal factors might play a role (10, 11),

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At a Glance Commentary

Scientific Knowledge on the

Subject: This study provides a complete analysis of airway wall structure and inflammation in preschoolers with severe recurrent wheeze.

What This Study Adds to the

Field: Markers of remodeling differ according to age; however, in the absence of control subjects we cannot distinguish the impact of disease on airway wall structure from that of age-related development. Airway wall structure is not related to inflammatory cell counts. Atopy and airway smooth muscle area are associated. Markers of remodeling are similar between multiple trigger wheezers and viral wheezers.

most of the postnatal loss of lung function seems to occur during the preschool years, with no subsequent recovery observed (12–15). Better knowledge of the structural changes in the airway wall in preschoolers with asthma may therefore help to prevent early loss of lung function and to modify the natural course of the disease.

A few studies have described airway remodeling in preschool children, mostly focusing on RBM thickness (16, 17) or ASM area (18). Saglani and coworkers (16) found no difference in RBM thickness between symptomatic infants younger than age 26 months with or without airflow obstruction. In another study, RBM thickness was found to be significantly greater in confirmed wheezers with severe recurrent symptoms (median age, 29 mo) than in nonwheezing age-matched control subjects (median age, 19.5 mo) (17). O'Reilly and coworkers (18) showed that ASM area had already increased in preschool wheezers (median age, 26 mo) who subsequently developed asthma at school age but not in those who did not develop asthma, whereas RBM thickness was similar between these groups.

These data suggest that remodeling, which was thought to be a consequence of long-standing airway inflammation, may actually occur early during the course of asthma, in parallel to ongoing inflammation and exacerbations rather than as a

consequence of them (2). The relationship between inflammation and remodeling in young children with wheeze remains poorly understood, with studies showing conflicting results (17, 19).

Patients with severe recurrent wheeze require evaluation, including bronchoscopy with bronchoalveolar lavage (BAL) and biopsies (20), and can therefore be assessed for airway remodeling and inflammation. The Severe Preschool Asthma Survey and Monitoring (SPASM) cohort was initiated in 2006, to describe airway wall structure and its relationship with inflammation and age in a well-defined population of preschoolers with severe recurrent wheeze confirmed by a doctor. The findings obtained were then compared with those previously described for schoolchildren with established severe asthma (5–12 yr) (9). We hypothesized that the morphometrics of the airways differ according to age in preschoolers with severe recurrent wheeze.

Methods

This study was approved by the institutional review board of Necker Hospital. Written informed consent was obtained from parents.

Subjects

Children aged 5 years or younger with recurrent wheeze were referred for further investigations. After the investigations, preschoolers with a doctor's diagnosis of severe recurrent wheeze were included. Symptoms were defined as wheezing and dry cough more than three times per week, or exacerbations requiring more than three oral corticosteroids burst or more than three emergency department visits during the previous 6 months despite optimal treatment for age (21). The preschoolers were assigned to two groups: group 1, children aged 36 months or less (Gr1); group 2, children aged 37–59 months (Gr2). Two patterns were identified (22): viral wheezers (VW) and multiple trigger wheezers (MTW). Biopsy results were then compared with those previously obtained in schoolchildren (Group 3 [Gr3], $n = 21$) with severe asthma (*see the online supplement*) (9).

Atopic Status

Atopy was defined as a positive standard skin prick test and/or specific IgE levels for

current allergens (*see the online supplement*) (23).

Bronchoscopy

Flexible bronchoscopy was performed under general anesthesia (24), with a 4-mm external diameter bronchoscope (BF/MP 160, Olympus, Hamburg, Germany). Biopsy samples were taken from the segmental bronchi of the right or left lower lobe (*see the online supplement*).

BAL and Tissue Processing

The first BAL aliquot was used for bacterial cultures, and immunofluorescence testing for respiratory viruses (*see the online supplement*) (25).

Tissue processing was described elsewhere (9). Four to six biopsy samples were taken. Bronchial biopsies with an altered structure were not used. The minimum size of the analyzed section was 0.1 mm^2 (range, $0.1\text{--}1 \text{ mm}^2$). The best sample was used for analysis. Sections were stained with May-Grunwald-Giemsa, and inflammatory cells were counted in the submucosa and expressed as number of cells per 0.1 mm^2 of submucosal area. We determined epithelial integrity as a percentage, and RBM thickness in micrometers. The percentage of the submucosal area occupied by mucus glands and ASM was determined on hematoxylin-eosin-stained and anti-SMA antibody (Dako)-treated sections. The bundles of ASM or mucus glands were enclosed with a line and the areas they covered were calculated by image analysis (1). Angiogenesis was assessed by anti-CD31 antibodies or isotype controls (Dako). The number of vessels stained with anti-CD31 mAb was determined for the area of at least one grid (0.1 mm^2) per biopsy, and is reported as the median number of positive sections per 0.1 mm^2 . Parameters were evaluated by two independent observers, blind to the study.

Results are expressed as the median of all measurements obtained for each patient (*see the online supplement*).

Statistical Analysis

Data are expressed as median (interquartile range). Differences between groups were assessed with the Mann-Whitney test or with the Kruskal-Wallis test, followed by a Mann-Whitney U test if a significant difference was found. Correlation analyses including data from the three age groups

were made using Spearman's rank correlation analysis. P less than 0.05 was considered significant. Data were analyzed with GraphPad Prism version 5.03 (GraphPad Software, Inc., San Diego, CA) (see the online supplement).

Results

Patients

Forty-nine preschoolers were included; 20 children were assigned to group 1 and 29 to group 2. The general characteristics of the preschoolers and the schoolchildren are summarized in Table 1. Twenty-eight preschoolers were VWs, and 21 were MTWs. Except for the number of exacerbations in the past 6 months, VWs and MTWs had similar general characteristics. Twenty-one preschoolers had atopy, 23 did not, and atopy status was unknown for five subjects. Preschoolers with and without atopy had similar characteristics (see Tables E1–E3 in the online supplement).

Airway Wall Structure According to Age

Figures 1–3 and Figures E1–E3 provide details regarding airway wall structure. Concerning parameters evaluated on different sections (RBM, epithelial integrity, ASM), the coefficient of variation for two sections from the same biopsy was 15%. For all the parameters, the variation between biopsies from the same patient was 11%. The interobserver reproducibility was higher than 0.85 including that for the semiquantitative data. The characteristics

of the airway wall for each group are summarized in Table 2.

RBM thickness. The RBM was significantly thinner in group 1 than in group 2 ($P = 0.02$). RBM thickness was strongly correlated to age ($P < 0.01$; $\rho = 0.62$), and was significantly higher in schoolchildren than in preschoolers ($P < 0.01$).

ASM area. ASM area did not differ between group 1 and group 2 ($P = 0.34$), whereas it was strikingly lower in preschoolers than in schoolchildren ($P < 0.01$). There was a moderate correlation between ASM area and age at biopsy ($P = 0.01$; $\rho = 0.31$). RBM thickness and ASM area were slightly positively correlated ($P < 0.01$; $\rho = 0.39$).

Vascularity. The number of vessels was higher in group 1 than in group 2 ($P = 0.02$), and in group 3 ($P < 0.05$). There was no relationship between the number of vessels and age at biopsy ($P = 0.07$; $\rho = -0.23$). Vascularity did not differ in preschoolers and schoolchildren ($P = 0.12$).

Mucus gland area. One value from a child in group 1, which was greater than the 99th percentile, was excluded from the analysis. Mucus gland area was similar in children in group 1 and group 2 ($P = 0.29$), and higher in group 2 than group 3 ($P < 0.05$). The area covered by mucus glands was unrelated to the age ($P = 0.05$; $\rho = -0.24$). The preschoolers had a larger mucus gland area than the schoolchildren ($P < 0.01$).

Epithelial integrity. Epithelial integrity was low in preschoolers, and did not differ between group 1 and group 2 ($P = 0.22$).

Epithelial integrity was not related to age ($P = 0.12$; $\rho = 0.20$). We found no differences in epithelial integrity between preschoolers and schoolchildren ($P = 0.25$).

Airway Inflammation

BAL. There was no difference in BAL cytology between group 1 and group 2 (Table 3). Microbiologic cultures were positive in 8 children in group 1 versus 11 in group 2 ($P = 0.89$). None of the children had a viral infection. We found no relationship between BAL cytology and airway wall structure (data not shown).

Cell counts in biopsy. Biopsies from children in group 1 contained more inflammatory cells than those from children in group 2 ($P = 0.03$), with fewer eosinophils ($P = 0.02$) (Table 4). Total cell counts in biopsy and in BAL were not correlated ($P = 0.68$; $\rho = 0.06$). Similarly, eosinophil and neutrophil counts in the biopsies were not correlated with those in BAL fluid ($P = 0.74$, $\rho = -0.04$ and $P = 0.49$, $\rho = 0.1$, respectively).

Cellular infiltration and airway wall structure. We found no relationship between inflammatory cell counts in biopsies and the morphometrics of the airway wall including vascularity (see Table E4).

Impact of Clinical Phenotypes on Airway Wall Structure and Inflammation

Atopy status. ASM area was significantly greater in preschoolers with atopy (13.1% [8–19%]) than in preschoolers without atopy (7.7% [3.2–10.7%]) ($P = 0.01$) (Figure 4). We found no differences in the other structural parameters (see Table E5 and Figure E4).

VWs and MTWs. Structural parameters did not differ between VWs and MTWs. We found no differences between VWs and MTWs regarding both the presence of pathogens in BAL fluid, and cellularity in BAL and biopsies (see Figures E5 and E6 and Tables E6 and E7).

Discussion

In this study, we quantified all the morphometric parameters associated with

Table 1. Clinical Characteristics of Preschoolers and Schoolchildren

	Preschoolers	Schoolchildren
Number	49	21
Age, mo	38.4 (29.3–49.5)	117.7 (102.4–124.3)
Sex, male/female	26/23	16/5
Atopic, n (%)	21 (43)	14 (67)
Sensitization to aeroallergens, n (%)	14 (67)	12 (57)
Sensitization to food allergens, n (%)	12 (57)	1 (5)
Parental atopy, n (%)	28 (57)	14 (67)
Duration of disease, mo	29.1 (5.2–56.4)	94.4 (85.8–107.7)
Regular inhaled steroids	49	21
No. of exacerbations in the last 6 mo	4 (3–6)	3 (2–4)
Inhaled steroids, $\mu\text{g/d eq. bdp}$	1,000 (500–2,000)	1,000 (800–1,000)

Definition of abbreviations: eq, bdp = beclomethasone equivalents; parental atopy = asthma and/or allergic rhinitis in at least one parent.

Data are expressed as the median (interquartile range).

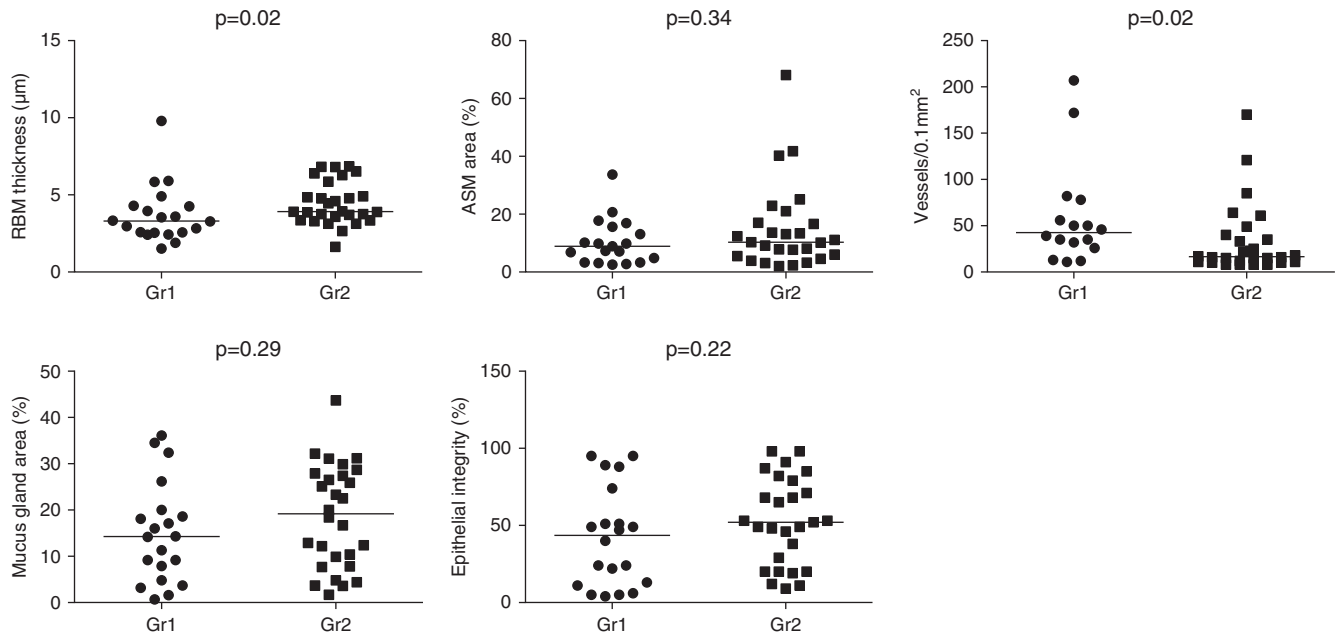


Figure 1. Airway wall structure according to age in preschoolers. Gr1 = group 1, ≤ 36 months, $n = 20$. Gr2 = group 2, children aged 37–59 months, $n = 29$. Reticular basement membrane (RBM) was thinner and vascularity was higher in Gr1 than in Gr2; the other parameters were similar. Missing data: one for RBM, three for airway smooth muscle (ASM), four for vascularity, and one for epithelial integrity and mucus gland.

airway wall remodeling in preschool children with severe recurrent wheeze, and analyzed whether they were related to inflammation. We demonstrate, in a highly selected population, that airway wall

structure and inflammatory cell counts are not related. We also found that ASM area is higher in preschoolers with severe recurrent wheeze with atopy than without atopy, and that airway wall structure and airway

inflammation are similar in VWs and MTWs. We observed differences in airway wall structure according to age. The younger preschoolers had a thinner RBM and a higher vascularity than the older

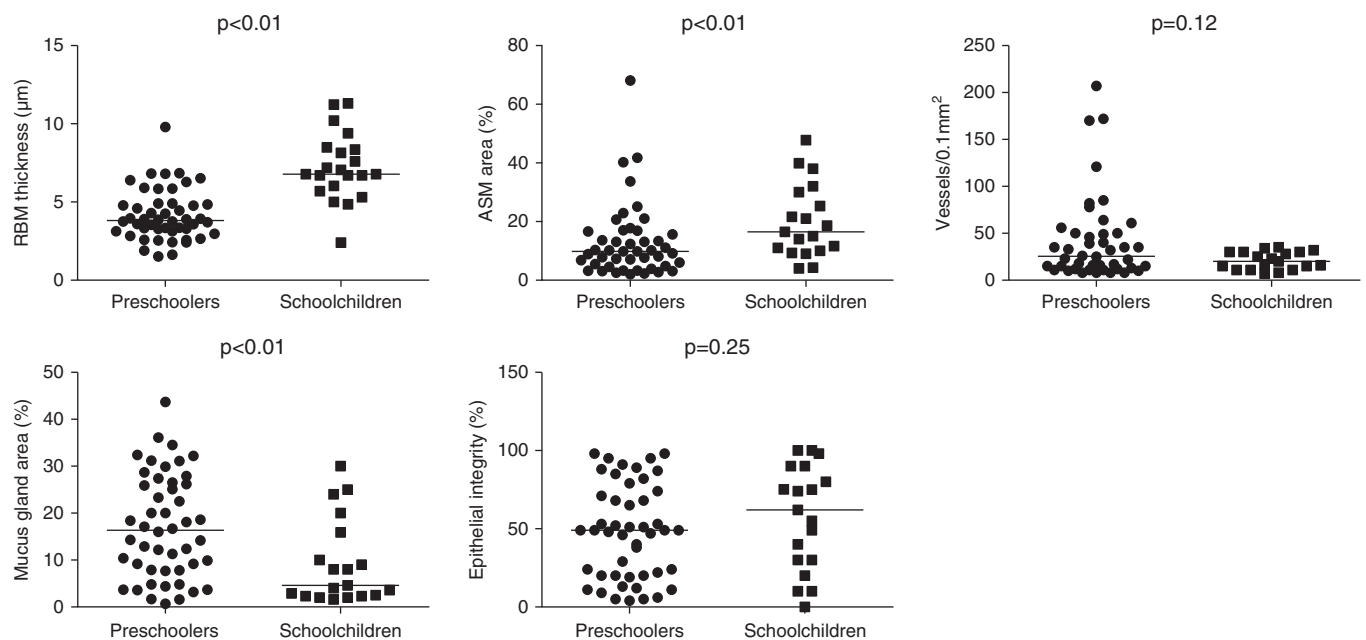


Figure 2. Airway wall structure in preschoolers and schoolchildren. Reticular basement membrane (RBM) thickness, airway smooth muscle (ASM), and mucus gland area differed between groups; the other parameters were similar. Missing data: one for RBM, five for ASM, five for vessels, and two for epithelial integrity and mucus glands.

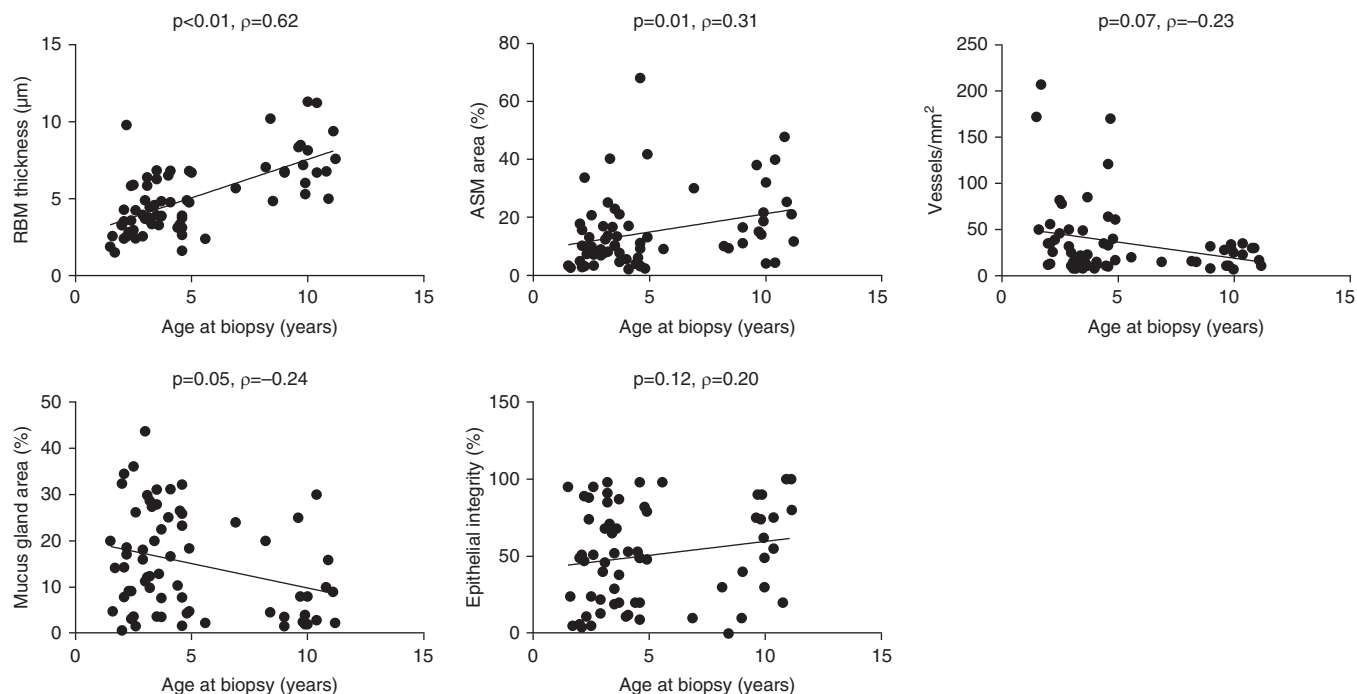


Figure 3. Correlation between airway wall structure and age. Age at biopsy was significantly positively correlated with reticular basement membrane (RBM) thickness and airway smooth muscle (ASM) area. Missing data: one for RBM, five for ASM, five for vessels, and two for epithelial integrity and mucus glands.

preschoolers. In addition, preschoolers with severe recurrent wheeze had a thinner RBM, a smaller ASM area, and a higher mucus gland area than school-aged children with severe asthma. However, in the absence of control subjects, we cannot determine whether differences observed in airway wall structure result from disease or normal age-related development (26).

The smaller number of eosinophils observed in group 1 versus group 2 is consistent with the finding that eosinophils are infrequent in biopsies from infants with

recurrent wheeze and/or cough (16, 27). Although biopsies from our younger preschoolers contained slightly more inflammatory cells than those from the older preschoolers, we found no relationship between airway wall structure and inflammatory cell counts in biopsies and in BAL. A correlation between eosinophil counts and RBM thickness was described in preschoolers with severe recurrent wheeze (17). Conversely, in a recent study, RBM thickness, vascularity, and epithelial loss were found to be similar

in children aged between 4.5 and 7.7 years with eosinophilic or noneosinophilic asthma (19). Differences between the phenotype of patients or the use of antiinflammatory therapies may account for these discrepancies. Treatment with high doses of inhaled corticosteroids (ICS) in adults with asthma is associated with a reduction in vessel number and vascularity (28, 29), and RBM thickness, ASM area, and the number of goblet cells (30). In our patients, we found no relationship between the dose of ICS and airway wall structure (data not shown). Altogether our findings suggest that, in our highly selected children, inflammation does not directly influence the markers of remodeling.

We found that preschoolers with severe recurrent wheeze with atopy, a group known to be at high risk of subsequently experiencing established asthma (31, 32), had a larger ASM area than preschoolers with severe recurrent wheeze without atopy. This finding may be related to the results of *in vitro* studies showing that ASM cells express a functional high-affinity IgE receptor (FcεRI) (33) and that the IgE sensitization of ASM cells directly induces ASM cell proliferation (34, 35).

Table 2. Characteristics of the Airway Wall by Age

	Group 1	Group 2	Group 3
Age, mo	27.2 (24.5–30.3)	44.5 (41–54.6)	117.7 (102.4–124.3)
Number	20	29	21
RBM thickness, μm	3.3 (2.6–4.3)* [†]	3.9 (3.5–5.1) [†]	6.8 (6–8.4)
ASM area, %	8.9 (4.1–14.4) [†]	10.3 (5.8–16.8)	16.5 (10.5–27.7)
Vessel number, per 0.1 mm^2	42.5 (30.5–61.5)* [†]	16.5 (11–36.3)	20 (13.30)
Mucus gland area, %	14.3 (7.1–19)	20 (5.8–16.8) [†]	4.6 (2.4–13)
Epithelial integrity, %	43.5 (12.5–56.8)	52 (24.5–75)	62 (30–85)

Definition of abbreviations: ASM = airway smooth muscle; RBM = reticular basement membrane.

Data are expressed as the median (interquartile range). Group 1: preschoolers less than or equal to 36 months. Group 2: preschoolers, 36–59 months. Group 3: schoolchildren.

* $P < 0.05$, versus group 2.

[†] $P < 0.05$ versus group 3.

Table 3. Bronchoalveolar Lavage Analysis in Preschoolers

	Group 1	Group 2	P Value
Number of patients	20	29	
Total cell counts	190 (135–250)	140 (100–205)	0.19
Neutrophils, %	32 (10–53)	10 (4–38)	0.10
Eosinophils, %	0 (0–1)	0 (0–1)	0.81
Lymphocytes, %	5 (1–8)	6 (1–8)	0.48
Macrophages, %	63 (42–82)	78 (61–87)	0.11
Viral infection	0	0	
Positive culture	8	11	0.89

Cell counts are expressed as number $\times 10^3$ /ml. Median (interquartile range).

Furthermore, the higher ASM mass in children with atopy may explain why children with atopic asthma have higher bronchial hyperresponsiveness than those with nonatopic asthma (36, 37). We found no difference in ASM area between the two groups of preschoolers, whereas ASM area was greater in school-aged children than in preschoolers. This confirms previous findings showing that ASM area is similar in preschool wheezers and control subjects (18), but it is larger in school-aged children with moderate-to-severe asthma than in control subjects (8).

Previous studies report that the number of vessels is higher in wheezy children (38) and in children with mild-to-moderate asthma (7) than in control subjects. In our study, the younger preschoolers exhibited a higher number of vessels than the older preschoolers. Although younger children are more prone to viral infections, we found no relationship between inflammatory cell counts in biopsies and vascularity. However, in the absence of control subjects, we cannot exclude the possibility that higher vascularity in the younger preschoolers reflects a physiologic process. Indeed, a higher rate of angiogenesis in younger children may be required to support the growth of the bronchial tree.

Our results are consistent with previous data showing that the RBM is thicker in school-aged children with severe asthma than in preschoolers with severe recurrent wheeze (16, 17). We also found that in severe preschool wheezers, RBM increases with age. However, based on post-mortem analysis of infants and children with no history of asthma who died from nonrespiratory causes, RBM thickness increases with age (26); therefore, it is difficult to determine whether the observed differences in RBM thickness between group 1 and group 2 are related to a normal or pathologic process. RBM thickness values cannot be compared directly between studies because of differences in patient phenotype and the location from which the biopsy specimens were taken in the airways; nevertheless the RBM thickness values obtained here were lower than those reported previously (7, 16, 17, 38). We also confirm that RBM thickness and ASM area are positively correlated (39, 40), suggesting that a common pathophysiologic mechanism affects both parameters.

We observed no mucus hypersecretion in our preschool subjects, but they had a larger mucus gland area than the school-aged children. Similar results have been

reported for individuals with difficult asthma (6–17 yr) (3), whereas high goblet cell numbers were observed in only 2 out of 10 schoolchildren (median age, 9.3 yr) with moderate asthma (4). Many factors contribute to goblet cell hyperplasia. Both damaged and virus-infected epithelia may produce mucus following epidermal growth factor receptor activation (41, 42). Thus, primary changes to the epithelium may contribute to the development of mucus glands (43).

There was a substantial overlap between preschoolers and school-aged children for all the parameters tested. In our opinion, this finding is a true reflection of the morphometrics of the airways in children with severe wheeze and supports the idea that changes in the airway wall occur progressively and that markers of remodeling may appear at different times from one child to another. However, it may also indicate the existence of different subphenotypes within each group as reported for atopy, or reflect differences in responsiveness to ICS. Regarding clinical phenotype, VW and MTW preschoolers with severe symptoms shared the same histologic and inflammatory features providing histologic evidence that the phenotypes can switch within patients (44).

Our study has several limitations. First, the main weakness is the absence of control subjects. Although generally well tolerated (45), endoscopic procedures are invasive and require general anesthesia. They are indicated in patients with persistent symptoms despite optimal treatment, to exclude an incorrect diagnosis of asthma. For ethical reasons, we did not perform any flexible bronchoscopy in asymptomatic children or in children with only mild symptoms (46). As mentioned above, the differences observed in RBM thickness and vascularity in our preschoolers may reflect a normal process and should be interpreted with caution. Second, we aimed to describe potential differences in airway wall structure according to age in preschoolers with severe recurrent wheeze. A longitudinal study involving multiple biopsies obtained from the same children would have been the best design. However, this was not feasible because of ethical reasons; thus, we performed a cross-sectional study including children ranging from 18 months to 5 years of age. Third, although all the patients were objectively assessed before inclusion, and

Table 4. Inflammatory Cell Counts in Biopsies from Preschoolers

	Group 1	Group 2	P Value
Number of patients	20	29	
Total cell count	373 (330–472)	339 (245–373)	0.03
Neutrophils	113 (52–215)	82 (46–125)	0.38
Eosinophils	6 (2–18)	14 (8–27)	0.02
Mononuclear cells	220 (186–300)	173 (120–251)	0.10

Cell counts are expressed as number per 0.1 mm². Median (interquartile range).

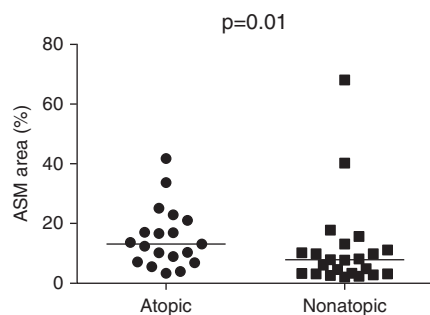


Figure 4. Airway smooth muscle (ASM) area is larger in preschoolers with severe recurrent wheeze with atopy than without atopy. There is one missing datum.

differential diagnoses of asthma were excluded, some of the patients included may not display asthma when they reach school age (44). Severe symptoms during

the preschool years seem to increase the risk of subsequent wheezing (44, 47); therefore, most of the patients studied would be expected to have wheezing at school age. The preschoolers studied here should be reassessed when they reach school age, to analyze their symptoms and lung function. Finally, our results were obtained in a group of children with severe symptoms and cannot be extrapolated to children with milder symptoms, even though remodeling has also been described in children with mild or moderate asthma (7, 8, 38).

In conclusion, this study demonstrates, in a well-defined population of preschoolers with severe recurrent wheeze, that airway wall structure is not correlated with inflammatory cell counts, and that atopy is associated with higher ASM area. This

supports the hypothesis that airway inflammation and remodeling may develop independently, and identifies smooth muscle as a therapeutic target in wheezy preschoolers with atopy. Finally, only studies including control subjects not suffering from any diseases of the airways would make it possible to determine whether the age-related changes observed in RBM thickness and vascularity reflect the onset of remodeling or a physiologic process. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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