Histopathology of Severe Childhood Asthma*

A Case Series

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Background: To date, little has been published describing the pathology of severe childhood asthma. The currently accepted model of asthma holds that persistent airway inflammation leads to various symptoms of asthma, airway hyperresponsiveness, and airway remodeling that ultimately results in permanent loss of lung function.

Methods: Evaluation of six children referred to the National Jewish Medical and Research Center with difficult-to-control asthma, despite aggressive anti-inflammatory therapy, who underwent bronchoscopy with endobronchial biopsy to better characterize their disease.

Results: In every case, endobronchial biopsies revealed changes consistent with airway remodeling characterized by thickening of the basement membrane, smooth-muscle hypertrophy, with varying degrees of goblet-cell and submucous gland hyperplasia. The degree of subbasement membrane thickening did not appear to correlate with baseline FEV₁, ultimate FEV₁ following aggressive therapy, or lability in lung function. In five of six cases, there was minimal to no histologic evidence for airway inflammation with mild and patchy submucosal lymphocytic infiltration noted; eosinophils and neutrophils were not present. Further, the majority of the patients achieved normal FEV₁ values despite significant subbasement membrane thickening, counter to the current beliefs regarding airway remodeling and irreversible loss of lung function.

Conclusions: This case report highlights some of the shortcomings of the current inflammatory paradigm for severe asthma. Despite little evidence of ongoing airway inflammation, many of the subjects displayed significant lung function lability. The lack of inflammation argues against steroid resistance at a cellular level, although it could be argued that inflammation may have been distal to the site sampled. Additionally, normal to nearly normal lung function was achieved despite the presence of significant remodeling. These findings suggest the need to look beyond inflammation to fully treat severe asthma and ultimately alter its progression.

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Key words: airway inflammation; airway remodeling; glucocorticoids; severe asthma

Abbreviations: BALF = BAL fluid; NJMRC = National Jewish Medical and Research Center

Our understanding of the pathophysiology of asthma has changed dramatically over the past several decades. The spectrum ranges from a completely reversible disorder limited to smooth-muscle dysfunction, to a complex and dynamic process involving the long-term effects of airway inflammation. As a result, therapies have shifted from treating the disease with bronchodilators alone, to an increased emphasis on treating the underlying inflammatory process. Yet, despite advances in our understanding and treatment of asthma, the morbidity and mortality of this disease has significantly increased over the last 20 years.1

The pathophysiology of chronic asthma is complex, in that it involves smooth-muscle dysfunction, acute and chronic inflammation, and structural changes within the airway, collectively termed airway remodeling.2,3 It has generally been assumed...
that airway inflammation is responsible for the various manifestations of asthma including shortness of breath, wheezing, bronchial hyperresponsiveness, smooth-muscle dysfunction and, ultimately, structural changes. However, inflammation is not the sole mediator of this disease. As effective as inhaled glucocorticoid therapy is, it alone is often insufficient to adequately control asthma in patients with moderate-to-severe persistent asthma. In addition, multiple studies have shown that doubling the dose of inhaled glucocorticoid in patients inadequately controlled on inhaled glucocorticoid therapy alone fails to provide significant improvement in efficacy, while increasing the potential for systemic and adverse effects. This unresponsiveness calls into question the paradigm that airway inflammation is solely responsible for the manifestations of asthma and that glucocorticoid-independent mechanisms need to be considered. Whether this reflects our limited understanding of the pathogenesis of airway remodeling and/or a lack of effective therapies to target glucocorticoid-insensitive mechanisms remain an open issue.

The literature describing the pathology of severe, steroid-dependent childhood asthma is virtually nonexistent. This case series is among the first to combine historical and clinical features, pulmonary physiology, and endobronchial biopsy results in a group of children with severe steroid-dependent asthma. All of the subjects studied had a long, if not lifelong history of severe, persistent, high-risk asthma refractory to aggressive management that included long-term oral and high-dose inhaled glucocorticoid therapy. A majority of these patients had been intubated at least once due to severe asthma exacerbations. In every case, the endobronchial biopsies revealed significant changes to the structure of the airways, with little to no airway inflammation present. These findings suggest that noninflammatory-mediated mechanisms may contribute to severe asthma in children.

Materials and Methods

Bronchoscopy and Biopsy Sample Processing

Flexible bronchoscopy was carried out using standard pediatric techniques. The safety of this procedure in severe asthmatics has been documented. For children < 12 years of age (depending on body size), the Olympus BF-3C20 (Melville, NY) [outer diameter, 3.7 mm] or the BF-3C40 (outer diameter, 3.6 mm) fiberoptic bronchoscopes were used; for adolescents, the Olympus BF-40 [outer diameter, 5.9 mm, suction channel 2.2 mm] was used. Patients underwent conscious sedation using IV narcotics (meperidine and fentanyl) and anxiolytics (midazolam) before and during the procedure. Local anesthesia was initially induced using nebulized 1% lidocaine and albuterol, 2 mL and 0.5 mL, respectively. Local anesthesia was maintained throughout the procedure by instillation of 1% lidocaine through the bronchoscope. Lidocaine was titrated to control discomfort and cough and was limited to no more than 5 mg/kg below the vocal cords to protect against lidocaine toxicity. Following sedation and anesthesia, flexible bronchoscopy was undertaken via the nasal route. Inspection of the entire upper and lower airway was done first. The scope was then wedged into a subsegment of the right middle lobe and BAL was carried out using 3 mL/kg total volume (maximum of 150 mL) of sterile normal saline solution in three separate aliquots. Each aliquot was hand aspirated into a large syringe and initially kept separate.

Finally, the bronchoscope was retracted into the lower trachea and the cup biopsy forceps was introduced. Three to six endobronchial samples were obtained from the mainstem carina and from several subsegmental carinae. Minor surface bleeding was encountered and did not require therapy with epinephrine. The scope was then retracted through the upper airway, and the procedure was terminated. The patient was monitored throughout the sedation until he/she was awake with stable vital signs and receiving oral fluids well. The specimens were processed as follows. The BAL fluid (BALF) was pooled if there was no visible blood contamination of any of the aliquots. If contaminated, the blood-tinged specimen was sent separately for microbiology. All specimens were sent for cell count and differential, and special stains for siderophages and lipid-laden macrophages. Stains and cultures were also obtained for bacteria, fungi, acid-fast mycobacteria, Legionella pneumophila, viruses, Chlamydia pneumoniae, and Mycoplasma pneumoniae. If indicated, a stain for Pneumocystis was also obtained.

The endobronchial specimens were coded and blindly examined by a single investigator (C.C.), preserved in formalin, processed, and embedded in paraffin. Routine histologic sections were cut at 5 µm and stained with hematoxylin-eosin. The presence of eosinophils was determined by their characteristic bilobed nucleus and eosin-staining granules within the cytoplasm. Pentachrome staining was also performed to better delineate the features of a remodeled airway. Specifically, each type of tissue stains a unique color. For example, smooth muscle stains red, collagen stains yellow, mucus stains turquoise, and elastin stains black. The thickness of the “total” basement membrane was assessed on two sections of the same biopsy sample (100 µm apart). Several estimates were made in each section stained with pentachrome, along its length at 200-µm intervals, and the values averaged. The measurements included both the true and reticular basement membrane. A semiquantitative scale was used to grade basement membrane thickness. A score of 3+ describes severe basement membrane thickening (10 µm), a score of 2+ describes moderate increase in basement membrane thickening (6 to 10 µm), while a score of 1+ describes mild thickening of the basement membrane (≤ 5 µm).

Results

Historical and Clinical Data

The cohort studied included six children (three whites and three African Americans) evaluated at National Jewish Medical and Research Center (NJMRC) from 1993 to 1998 for evaluation of severe, persistent, steroid-dependent asthma and who underwent a clinically indicated bronchoscopy with endobronchial biopsy. The decision to perform bronchoscopy was at the discretion of the attending physician. The bronchoscopies were performed in every case to rule out other respiratory conditions.
and/or to evaluate for the presence inflammation despite aggressive inhaled and systemic glucocorticoid therapy. The ages of the study cohort at the time of evaluation ranged from 6 to 17 years (mean, 11.2 years), and the mean age of disease onset was 1.8 years. Four of the six patients had been intubated at least once due to a respiratory arrest secondary to a severe asthma exacerbation. All were receiving oral glucocorticoids at the time of admission (mean daily dose, 21 mg) and had required long-term oral glucocorticoid therapy for a mean of 5.2 years (Table 1). All subjects underwent an extensive diagnostic evaluation. Three of the six subjects required an increase in their oral glucocorticoid therapy to optimize lung function and minimize diurnal variability. Four subjects had significant airflow obstruction with hyperinflation and air trapping (Table 2), while two subjects had very little evidence for airflow obstruction or diurnal variability throughout their hospital stay. At the time of discharge, a significant improvement in lung function was noted in every child, with the mean FEV1 increasing from 69.5 to 100.8% of predicted (Table 2).

Review of the endobronchial specimens revealed evidence for significant structural alterations compatible with the current definition of airway remodeling in every subject studied. Specifically, grossly thickened subbasement membranes, goblet-cell hyperplasia, smooth-muscle hypertrophy and hyperplasia, and varying degrees of both elastin deposition and epithelial desquamation were noted. Of some surprise was the very obvious lack of airway inflammation. In five of the six cases, there was only mild and patchy lymphocytic inflammation immediately below the subbasement membranes, with few to no eosinophils or neutrophils noted.

**Case Summaries**

**Case 1:** A 9-year-old boy developed respiratory symptoms at 10 months following an episode of bronchiolitis. He was hospitalized numerous times for acute asthma exacerbations, with one episode requiring intubation. In addition, he had been receiving long-term oral glucocorticoids since 15 months of age. The hospital admission prednisone dose was 10 mg on alternate days in addition to high-dose inhaled glucocorticoids, and he required 6 to 10 inhalations of albuterol per day for symptoms. Spirometry initially revealed significant diurnal variability in his lung function, with wheezing and significant airflow obstruction on awakening (Fig 1, top, A).

At NJRMC, a dramatic reduction in diurnal variability was noted that was likely secondary to allergen avoidance, improved medication adherence, and im-
proved inhaler technique. His prednisone was successfully tapered to 7.5 mg on alternate days with no deterioration in symptoms or diurnal variability (Fig 1, top, A). On bronchoscopy, the airway anatomy was grossly normal. The BALF and culture findings were unremarkable. Endobronchial biopsy samples (Table 3) showed a greatly thickened basement membrane, massive smooth-muscle hypertrophy, and goblet-cell hyperplasia (Fig 2). In addition, a large submucous gland was noted, as was mucus lining the epithelium. There were only scattered eosinophils submucosal lymphocytes noted. Despite the histologic evidence for significant airway remodeling, the patient attained normal lung function at the time of discharge.

Case 2: A 12-year-old African-American girl had a long history of severe, persistent asthma beginning at 14 months. She was hospitalized numerous times, and had required intubation and mechanical ventilation three times for acute severe asthma exacerbations. Long-term prednisone was required since the age of 8 years (Table 1). On hospital admission, medications included 10 mg of prednisone administered on alternate days, high-dose inhaled glucocorticoids, and theophylline. Her evaluation is summarized in Table 2.

Serial spirometry revealed diurnal variation in FEV1 values ranging from 75 to 103% of predicted (Fig 1, center, B). A prednisone burst resulted in a significant reduction in diurnal variability. Bronchoscopy with endobronchial biopsy was performed after 2 weeks of daily prednisone. Airway anatomy was normal while the airway mucosa was markedly inflamed and friable. The BALF cell differential was abnormal, with a substantially increased percentage of neutrophils (22%; normal, 1.5 to 2.1%) and lymphocytes (28%; normal, 8.9 to 10.1%) with an accompanying reduction in the percentage of macrophages (50%; normal, 87 to 89%); no eosinophils were present. Biopsy showed a partially denuded epithelium, squamous metaplasia, goblet-cell hyperplasia, markedly thickened subbasement membrane, and a significant quantity of elastin fibrils immediately below the basement membrane (Fig 3, Table 3).

Case 3: An 8-year-old girl with a long history of severe, persistent, steroid-dependent asthma, developed respiratory symptoms at 10 months with a severe disease course since, requiring numerous hospitalizations and three episodes requiring intubation and mechanical ventilation. In addition, oral glucocorticoids were regularly administered since the age of 6 years, and frequent glucocorticoid bursts were required since the age of 1 year. Airway anatomy was normal with a minimally inflamed
respiratory epithelial mucosa. The endobronchial biopsy revealed evidence for mild eosinophilic inflammation (Table 3).

Case 4: A 6-year-old girl with severe, persistent, steroid-dependent asthma developed respiratory symptoms at the age of 18 months. She required several hospitalizations and long-term oral glucocorticoid therapy since the age of 2 years. Medications on hospital admission included 10 mg of prednisone on alternate days, high-dose inhaled glucocorticoids, and theophylline. A prednisone burst was instituted secondary to significant diurnal variability (Fig 1, bottom, C) with FEV1 values on awakening consistently ≤ 50% of predicted. Despite a 6-day course of high-dose prednisone, lung function failed to significantly improve. A pharmacokinetic study found rapid prednisolone clearance. Methylprednisolone, 20 mg bid, was administered. After 30 days of high-dose, daily oral glucocorticoid therapy, her FEV1 values slowly improved, yet airflow obstruction persisted with FEV1/FVC ratios consistently < 0.80 (Fig 1, bottom, C).

Bronchoscopy with endobronchial biopsy was performed at the end of week 4. Airway anatomy was normal, while the airway mucosa appeared edematous. The BALF was unremarkable, while the endobronchial biopsy (Table 3) showed an intact but hyperplastic epithelium, significant squamous metaplasia, smooth-muscle hyperplasia, and a markedly thickened basement membrane (Fig 4). No airway inflammation was noted.

Case 5: A 15-year-old African-American girl had a long history of severe, persistent, steroid-dependent asthma, atopic dermatitis and respiratory symptoms developed at 2 years of age. She had mild, intermittent asthma until she was 11 years old. At that point, she required numerous hospitalizations and long-term oral steroids. Serial spirometry revealed significant diurnal variation in FEV1 values, which decreased following an oral glucocorticoid burst. Endobronchial biopsy specimens (Table 3) showed marked bronchial basement membrane thickening with squamous metaplasia, goblet-cell hyperplasia, dense, irregular deposition of elastin fibers, and a mild eosinophilic inflammatory infiltrate.

Case 6: A 16-year-old African-American boy with severe, persistent, steroid-dependant asthma was first diagnosed at 4 years of age. The asthma significantly worsened; from 11 to 16 years, numerous hospitalizations were required with several episodes of loss of consciousness, and he was intubated three times for respiratory failure. His asthma was characterized by significant nocturnal symptoms, and diur-

### Table 3—Endobronchial Biopsy Results*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Inflammatory Infiltrate</th>
<th>Basement Membrane Thickness†</th>
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<th>Goblet-Cell Hyperplasia</th>
<th>Submucosal Gland Hyperplasia</th>
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<td>−</td>
<td>3+</td>
<td>+</td>
<td>+</td>
<td>N/A§</td>
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* = absent; + = present; N/A = not available.

† Semiquantitative scale: 1+ is a mild increase in thickness (≤ 5 μm), 2+ is a moderate increase (6–10 μm), and 3+ is a severe increase in thickness (> 10 μm).

‡ Scattered eosinophils, lymphocytes.

§ Depth of endobronchial biopsy inadequate to assess bronchial smooth-muscle layer or submucosal glands.
nal variability in peak flows. At 16 years of age, he had a respiratory arrest and died.

By the age of 11 years, he became steroid dependent, requiring 20 to 25 mg of prednisone on alternating days as well as monthly bursts of prednisone to control exacerbations. Serial spirometry revealed significantly obstructed lung function, with FEV$_1$ values initially ranging from 45 to 60% of predicted. His FEV$_1$ slowly and modestly improved with an increase in prednisone dose, but the response was never complete (Table 2). Initial endobronchial biopsy samples (Table 3) revealed significant basement membrane thickening, smooth-muscle hypertrophy, and clusters of neutrophils within the lamina propria. In addition, there was evidence for significant edema within the lamina propria (Fig 5). BALF cell counts showed moderate lipid-laden macrophages with an index of 76 (high = 100). A repeat biopsy 7 months later showed similar findings.

Between the ages of 11 years and 13 years, this patient continued to have poorly controlled asthma despite high-dose oral glucocorticoids and required multiple hospitalizations. A third bronchoscopy was performed at the age of 16 years. As noted in the previous biopsies, there was marked thickening of the basement membrane with mature and immature collagen deposition and smooth-muscle hypertrophy and hyperplasia. Only occasional neutrophils and eosinophils were present in the fibrotic and edematous submucosa.

**Discussion**

Airway remodeling is a distinctive pathologic feature of asthma,¹ and is thought to be the result of an aberrant reparative process associated with ongoing allergic inflammation. The histopathologic changes that occur within the airways of asthmatics include epithelial desquamation and regeneration, goblet-cell hyperplasia, submucosal gland hypertrophy, subepithelial fibrosis or thickening of the basement membrane, inflammatory cell infiltration, hyperplasia and hypertrophy of the bronchial smooth muscle, and vascular changes.⁸ What role these changes have on asthma severity and disease course remains to be determined.

This case series is among the first to combine historical and clinical features, pulmonary physiology, and endobronchial biopsy results in a group of children with severe asthma. All of the subjects studied had long, if not lifelong, histories of severe, persistent, high-risk asthma, with the majority having been intubated at least once for a severe asthma exacerbation. In addition, all required long-term oral and inhaled glucocorticoid therapy, yet adequate asthma control had been difficult to achieve. During their evaluation, all of the subjects displayed improvement in lung function, with the mean FEV$_1$ increasing from 69.5 to 100.8% of predicted (Table 1). Despite structural alterations of the airways, compatible with airway remodeling, normalization of lung function was nonetheless achievable in nearly every subject. In addition, there was a noticeable lack of airway inflammation noted on endobronchial biopsy, perhaps the result of aggressive glucocorticoid therapy. However, the absence of inflammation may indicate that airway inflammation need not be a prominent feature of refractory asthma.

The observations from this cohort of children provide insight into the pathophysiology of severe childhood asthma. First, significant airway remodel-
ing can occur in children with severe asthma as young as 6 years old. Second, airway remodeling occurred despite aggressive, long-term treatment with both systemic and inhaled glucocorticoids. Third, in five of the six patients studied, there was little to no airway inflammation. It is possible that the

Figure 3. Endobronchial biopsy from case 2. This pentachrome-stained slide (original × 20) demonstrates a partially denuded epithelium, squamous metaplasia, and goblet-cell hyperplasia (stains turquoise), markedly thickened basement membrane (stains yellow), and deposition of elastin fibrils (stains black) immediately below the basement membrane.

Figure 4. Endobronchial biopsy from case 4. This hematoxylin-eosin–stained slide (original × 20) demonstrates an intact but hyperplastic airway epithelium and markedly thickened basement membrane, with only minimal inflammation characterized by patchy lymphocytic infiltration immediately below the basement membrane.
high-dose glucocorticoid therapy administered in a controlled setting was sufficient to suppress any active eosinophilic inflammation, but the absence of neutrophils, generally refractory to glucocorticoid, was striking. Whether systemic glucocorticoid therapy was insufficient to inhibit inflammation, which eventually resulted in airway remodeling seen in these children, cannot be answered at present. Alternatively, airway remodeling may have occurred independently of airway inflammation, and in a glucocorticoid-insensitive manner. Fourth, the structural abnormalities noted on endobronchial biopsy did not appear to predict the clinical response, at least while at NJMRC. For example, case 4 required > 30 days of high-dose oral glucocorticoid therapy to achieve nearly normal lung function, while the lung function in case 5 improved to the normal range despite a complete reduction in the oral glucocorticoid dose in the face of similar findings on bronchial biopsy. Lastly, despite findings consistent with significant airway remodeling (basement membrane thickening and smooth-muscle hypertrophy/hyperplasia), the majority of the patients, by the time of discharge, achieved normal FEV₁ values associated with an accompanying decrease in diurnal variability. These observations argue against the concept that a grossly thickened basement membrane results in irreversible airflow obstruction, at least among children with severe asthma.

Our findings contrast with those of Chetta et al,9 who found the degree of basement membrane thickness to be associated with asthma severity in adults based on symptoms. Another study by the same group found statistically significant inverse correlations between basement membrane thickness and baseline FEV₁, and provocative concentration of methacholine causing a 20% fall in FEV₁ values, and a direct correlation with daily peak expiratory flow variability in adults with asthma.10 As compelling as these studies may appear, neither our results nor those of other investigators have confirmed such associations. For example, Wenzel et al11 studied a well-defined group of patients with mild, moderate, and severe asthma, and found no statistical difference in basement membrane thickness among the groups they studied.

This case series is obviously limited by sample bias, and small sample size precluding simple generalizations. In addition, we cannot comment on whether our subjects had elevated numbers of mast cells within their airways, as Giemsa staining was not performed. With that said, mast cells were not found to be elevated in the study by Wenzel et al,11 who found no difference between the number of mast cells within the airways of severe asthmatics compared to mild asthmatics and control subjects. Children with asthma referred to NJMRC often are among the most severe. In addition, only a minority of the children referred to NJMRC undergo bronchoscopy with biopsy. Nevertheless, these cases are illustrative of the complexity of asthma and the inability to make simple structure-function correlations. The data suggest that airway inflammation may not be the sole mediator in the histopathology of asthma; any number of inflammation-independent factors might contribute. Review of the endobronchial biopsy specimens of each case revealed significant airway remodeling largely in the absence of significant airway inflammation. Of concern, these structural changes occurred despite long-term administration of high-dose inhaled and systemic glucocorticoid therapy. These observations demonstrate that extensive airway remodeling can be present in children with severe asthma, despite aggressive anti-inflammatory therapy. As such, research must be directed at better understanding the mechanisms involved in airway remodeling in children with severe asthma.

**Figure 5.** Endobronchial biopsy from case 6. This hematoxylin-eosin–stained slide (original × 20) demonstrates a massively thickened basement membrane, significant submucosal edema, and mild lymphocytic infiltration within the lamina propria.
involved in airway structural changes and remodeling. Only when these mechanisms are elucidated can effective therapies be designed. It is likely that glucocorticoid-independent and glucocorticoid-insensitive mechanisms play a prominent role in these pathologic processes, further emphasizing the need for novel therapeutic approaches.

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REFERENCES