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Pediatric severe asthma is characterized by eosinophilia and remodeling without T_H2 cytokines

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Abstract

Background—The pathology of pediatric severe therapy-resistant asthma (STRA) is little understood.

Objectives—We hypothesized that STRA in children is characterized by airway eosinophilia and mast cell inflammation and is driven by the T_H2 cytokines IL-4, IL-5, and IL-13.

Methods—Sixty-nine children (mean age, 11.8 years; interquartile range, 5.6-17.3 years; patients with STRA, n = 53; control subjects, n = 16) underwent fiberoptic bronchoscopy, bronchoalveolar lavage (BAL), and endobronchial biopsy. Airway inflammation, remodeling, and BAL fluid and biopsy specimen T_H2 cytokines were quantified. Children with STRA also underwent symptom assessment (Asthma Control Test), spirometry, exhaled nitric oxide and induced sputum evaluation.

Results—Children with STRA had significantly increased BAL fluid and biopsy specimen eosinophil counts compared with those found in control subjects (BAL fluid, $P < .001$; biopsy specimen, $P < .01$); within the STRA group, there was marked between-patient variability in eosinophilia. Submucosal mast cell, neutrophil, and lymphocyte counts were similar in both groups. Reticular basement membrane thickness and airway smooth muscle were increased in patients with STRA compared with those found in control subjects ($P < .0001$ and $P < .001$, respectively). There was no increase in BAL fluid IL-4, IL-5, or IL-13 levels in patients with STRA compared with control subjects, and these cytokines were rarely detected in induced sputum. Biopsy IL-5^+ and IL-13^+ cell counts were also not higher in patients with STRA compared with those seen in control subjects. The subgroup (n = 15) of children with STRA with detectable BAL fluid T_H2 cytokines had significantly lower lung function than those with undetectable BAL fluid T_H2 cytokines.

Conclusions—STRA in children was characterized by remodeling and variable airway eosinophil counts. However, unlike in adults, there was no neutrophilia, and despite the wide

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range in eosinophil counts, the T_H2 mediators that are thought to drive allergic asthma were mostly absent.

Keywords

Pediatric asthma; eosinophilia; remodeling; severe therapy-resistant asthma; mediators

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The 5% of patients with asthma classified as severe consume more than 50% of health care resources for the disease. Previous pediatric studies have investigated the pathology of "difficult asthma"[1,2] after a steroid trial and also mild-to-moderate asthma,[3,4] but the pathology of true severe therapy-resistant disease has been little studied. Children referred to us for evaluation of symptoms refractory to high-dose conventional asthma therapy are considered to have "problematic severe asthma."[5] This group comprises those with the wrong diagnosis, asthma with comorbidity, difficult asthma, and severe therapy-resistant asthma (STRA). They undergo detailed evaluation both at home and in the hospital.[5,6] Those with difficult asthma in whom potentially reversible factors (persistent allergen exposure, poor adherence, and poor inhaler technique) contributing to poor asthma control are identified are excluded.[5] Those remaining with genuine STRA undergo invasive testing with bronchoscopy, bronchoalveolar lavage (BAL), and endobronchial biopsy to characterize airway pathology and develop an individualized treatment plan.[7]

Previous studies have reported submucosal eosinophilia in pediatric patients with mild-to-moderate asthma[3,4] but not in children with problematic severe asthma.[2] However, this included patients with difficult asthma and subjects in whom bronchoscopy was performed after a high-dose steroid trial. A further report in patients with difficult asthma has shown increased epithelial eosinophil and neutrophil counts and higher BAL levels of IFN-γ in children with persistent symptoms compared with those with good symptom control.[8] However, there was no nonasthmatic control group and no assessment of basic asthma management.

In summary, the basic mechanisms and airway pathology in children with true STRA are unknown. Adult studies suggest that in many patients the pathobiology of severe asthma is mediated by immune pathways driven by T_H2-type $CD4^+$ T cells, with signature cytokines including IL-4, IL-5, and IL-13.[9,10] Hence biological modifiers of T_H2-type cytokines are a rational strategy for developing new treatment approaches in adults.[9] However, all studies to date, whether *in vivo* experimental models or *in vitro* models, have been conducted with adult animals[11] or samples,[12] respectively. Children with STRA are known to be markedly atopic,[13,14] and therefore we hypothesized that these children would also have eosinophilic and mast cell inflammation and that childhood STRA would be characterized by increased levels of the T_H2 cytokines IL-4, IL-5, and IL-13. The aim of this study was to characterize the pathology and mediators of inflammation and remodeling in patients with STRA.

METHODS

Subjects

Children aged 5 to 16 years referred to Royal Brompton Hospital with problematic severe asthma between April 2005 and June 2009 were included.[6] Definitions of poor control and entry criteria were as follows:

1. persistent (most days for ≥3 months) chronic symptoms (which prompt use of short-acting β_2-agonists ≥3 times per week) of airway obstruction despite high doses of inhaled corticosteroids (ICSs; ≥800 μg/d budesonide equivalent) and/or

regular oral corticosteroids, long-acting β_2-agonists, and current (or previous failed trial of) montelukast OR

2. recurrent severe exacerbations requiring 1 or more admission to the intensive care unit or 2 or more hospital admissions requiring intravenous medications or 2 or more courses of oral corticosteroids in the past year despite therapy for persistent symptoms as described in (1) above OR
3. at least 1 very sudden (≤6 hours) severe attack (requiring hospitalization) without warning despite therapy for persistent symptoms, as described in (1) above.

Outpatient nurse-led assessments were performed to assess medication, dose, device and technique, atopic status, and asthma understanding. A home and school visit was undertaken to assess adherence, the environment, and any psychosocial issues. Evaluation of adherence included prescription records, accessibility of medications, and whether medications were in date and/or unwrapped; allergen sources, such as pets within the home; and environmental tobacco smoke exposure (including cotinine measurements).

After assessment,[6] those reclassified as having difficult asthma[5] were excluded (Fig 1). The remaining patients with STRA were further investigated. The study was approved by the local research ethics committee, and all procedures were performed after obtaining written informed parental consent and, where appropriate, child assent.

Nonasthmatic control subjects (aged 6-16 years) were either (1) undergoing a bronchoscopy to investigate upper airway symptoms and agreed to extra research samples being taken or (2) were undergoing general anesthesia for cardiac catheterization and agreed to have a research bronchoscopy at the same time. They had no history of wheeze or lower airway symptoms, and all were deemed sufficiently stable that the extra few minutes to take research samples[15] were permissible, as judged by an anesthetist independent of the study. The clinical characteristics of control subjects are shown in Table E1 in this article's Online Repository at www.jacionline.org.

Investigations

All investigations were performed on the same day. None of the children had an exacerbation at the time of bronchoscopy, and at least 2 weeks were required between the last exacerbation and investigations. Details of investigations are given in the Methods section in this article's Online Repository at www.jacionline.org.

Atopic status—Atopy was defined as 1 or more positive specific IgE RAST (≥0.34 kU/L) or 1 or more positive skin prick test results to aeroallergens.

Symptom control—Asthma control was assessed by using the Asthma Control Test[16,17]; poor control was defined as a score of less than 20 of 25 (see Fig E1 in this article's Online Repository at www.jacionline.org).

Lung function—Spirometry and bronchodilator reversibility (BDR) defined as a greater than 12% change from baseline FEV_1 were measured according to American Thoracic Society/European Thoracic Society guidelines.[18,19]

Exhaled nitric oxide—Exhaled nitric oxide measurements at a flow rate of 50 mL/s were made with a chemiluminescence analyzer (NIOX; Aerocrine AB, Solna, Sweden) in accordance with American Thoracic Society/European Thoracic Society guidelines.[20]

Sputum induction and processing—Sputum induction was performed with 3.5% saline (0.9% if postbronchodilator FEV_1 was <65% of predicted value).[21] Samples were processed by adding 0.1% dithiothreitol (DTT). Supernatants were stored at −80°C. Samples were considered adequate if 80% or fewer squamous cells and 400 or more inflammatory cells were present and distinguishable.

Bronchoscopy, BAL, and endobronchial biopsy—Bronchoscopy, BAL, and endobronchial biopsy were performed under general anesthesia, as previously described.[22] BAL fluid was processed for cytology by the hospital cytopathology department. Eosinophilia was defined as greater than 1.19%[23] and neutrophilia as greater than 3.5%[24] of the total cell count. Paraffin-embedded biopsy specimens were cut and stained with hematoxylin and eosin (H&E) to assess quality and adequate morphology and also to quantify airway remodeling (reticular basement membrane [RBM] thickness,[25] epithelial shedding,[4] and airway smooth muscle [ASM] mass[26]). Evaluable sections were also stained for eosinophils (Congo red dye), neutrophils (neutrophil elastase), mast cells (mast cell tryptase), and $CD45^+$, $CD4^+$, and $CD8^+$ cells (for more information, see the Methods section in this article's Online Repository). All sections were coded, and inflammatory cells were expressed per square millimeter of tissue.[25]

Induced sputum, BAL fluid, and biopsy specimen cytokine analysis—Cytokines were quantified in BAL fluid and sputum supernatants by using the Luminex multiplex bead analysis system. The premade 21-plex Luminex plate (Bio-Rad Laboratories, Hercules, Calif) was used to assay levels of IL-1β, IL-4, IL-5, IL-8, IL-13, eotaxin, and IFN-γ. In addition, the Cytometric Bead Array (CBA Human Inflammation kit; BD Biosciences, PharMingen, San Diego, Calif) was used to quantify IL-5 and IL-13 (for more information, see the Methods section in this article's Online Repository).[27] Endobronchial biopsy specimens were stained for $IL\text{-}5^+$ and $IL\text{-}13^+$ cells by using immunohistochemistry, and positive submucosal cells were quantified and expressed per square millimeter of tissue (for more information, see the Methods section in this article's Online Repository).

Additional investigations to exclude alternate diagnoses—All children in whom BDR could not be demonstrated or who were nonatopic underwent high-resolution computed tomographic chest scans to exclude diagnoses, such as bronchiectasis or obliterative bronchiolitis. They also underwent blood tests to exclude rheumatologic diseases and pH studies to exclude gastroesophageal reflux.

Statistical analysis

There are insufficient data to inform a power calculation for pediatric biopsy studies; adult studies have reported that groups of 11 to 17 are sufficient.[25] The sample size for control subjects was opportunistic. Differences between groups were assessed by using the Mann-Whitney *U* test. Associations were assessed with Spearman correlation. Intraobserver repeatability was measured by calculating the coefficient of variation (CV) of 3 measurements of a section made on 3 occasions at least 3 days apart. Within-subject variability was assessed based on the CV of counts from 3 or more biopsy specimens from the same subject. Analysis was performed with the SPSS software, version 17 (SPSS, Inc, Chicago, Ill). A *P* value of less than .05 was considered significant for between-group comparisons, and a *P* value of less than .01 was considered significant for correlations.

RESULTS

Demographics

Of 104 patients referred with problematic severe asthma, 51 were classified as having difficult asthma (Fig 1). Fifty-three (51%) of 104 patients with true STRA were further investigated. The clinical characteristics of subjects are shown in Table I. Forty-five (85%) of 53 patients with STRAwere atopic compared with 7 (47%) of 15 control subjects ($P <$. 01). The median baseline FEV_1 in patients with STRA was 68.5% (interquartile range [IQR], 54.8% to 86.5%) of predicted value compared with 94% (IQR, 85% to 106%) in control subjects ($P <$ .0001).

Bronchoscopic findings

There were no complications after bronchoscopy. Findings from nonasthmatic control subjects are listed in Table E1. Positive BAL fluid bacterial cultures were present in 10 (19%) of 53 patients with STRA, of whom 7 (70%) of 10 also had BAL fluid neutrophilia. Positive cultures and neutrophilia were present in 2 control subjects. BAL fluid supernatants from patients with a positive bacterial culture and neutrophilia were excluded from cytokine analysis.

Inflammation in BAL fluid and biopsy specimens

Although BAL fluid and biopsy specimens were taken from all subjects, not all samples were of sufficient quality for analysis. One hundred twenty-one biopsy specimens were taken from 69 subjects, and at least 1 specimen was suitable for assessment for all subjects, except 1 control child. Three patients with STRA and 4 control subjects only had evaluable H&E-stained sections and therefore only had quantification of airway remodeling. The CV for intraobserver repeatability for all biopsy specimen inflammatory cells and cytokines was less than 10% (see Table E2 in this article's Online Repository at www.jacionline.org). The intraobserver repeatability for RBM thickness, epithelial shedding, and oral corticosteroid subepithelial volume fraction of ASM indexed to subepithelial tissue (Vv) smooth muscle were 4%, 6.4%, and 11.6%, respectively. The within-subject between-biopsy variability for eosinophil counts was 54% (see Table E3 in this article's Online Repository at www.jacionline.org). However, the between-subject variation in biopsy specimen eosinophil counts for patients with STRA was 185%.

BAL fluid was available in 50 of 53 patients with STRA and 14 of 16 control subjects (Fig 1). Children with STRA had significantly increased BAL fluid eosinophil counts compared with those seen in control subjects (median, 2.7% [IQR, 1% to 7.7%] vs 0% [IQR, 0% to 0.9%], respectively; $P <$.001), although there was wide variability among patients with STRA (Fig 2, A). Subepithelial mucosal eosinophil counts were significantly higher in patients with STRA than in control subjects (median, 11.2/mm^2 [IQR, 0-33.7/mm^2] vs 0/mm^2 [IQR, 0-25.1/mm^2], respectively; $P <$ .01; Fig 2, B-D).

There were no group differences in BAL fluid neutrophil or lymphocyte counts, submucosal neutrophil counts, mast cell counts, and $CD45^+$, $CD4^+$, or $CD8^+$ cell counts (Table II). Intraepithelial and smooth muscle inflammatory cells were similar between groups. No associations were found between mucosal inflammation and lung function, sex, intubation for asthma, persistent airflow limitation, gastroesophageal reflux, or serum IgE levels.

BAL fluid and sputum cytokine levels

There were no significant differences between patients with STRA and control subjects in levels of BAL fluid IL-1β, IL-4, IL-5, IL-8, IL-13, IFN-γ, and eotaxin when measured with the Luminex multiplex assay (see Table E4 in this article's Online Repository at

www.jacionline.org). A few children with STRA had detectable levels of BAL fluid IL-4 (n = 10), IL-5 (n = 8), and IL-13 (n = 8); however, the group median value was in the undetectable range (see Table E4). To confirm these findings, BAL fluid IL-5 and IL-13 levels were also quantified by using the Cytometric Bead Array, and again, there was no difference between patients with STRA and control subjects (see Table E4). The limits of detection for cytokines used in both assays are shown in Table E5 in this article's Online Repository at www.jacionline.org.

There was no difference in BAL fluid T_H2 cytokine levels between children with persistent symptoms and those with frequent exacerbations. However, children who had any detectable BAL fluid T_H2 cytokines (any of IL-4, IL-5, or IL-13) had significantly lower FEV_1 and forced vital capacity (FVC) percent predicted values and greater BDR than those with undetectable T_H2 cytokines (Table III).

Forty-one children underwent sputum induction, and 36 had a sufficient sample. Children with STRA, as a group, had undetectable levels of IL-4, IL-13, and IFN-γ in sputum (see Table E4). Eight children with STRA had detectable levels of sputum IL-5 (median, 0.9 pg/mL [IQR, undetectable-1.0 pg/mL]).

Submucosal IL-5$^+$ and IL-13$^+$ cells

Tissue expression of IL-5 and IL-13 was assessed to further support findings from the BAL fluid and sputum cytokine quantification. The intraobserver repeatability of biopsy specimen cytokine-positive cells was less than 10%. The within-subject, between-specimen variability for biopsy specimen IL-5 expression was 39%, and the between-subject variability in biopsy specimen IL-5 expression for control subjects was 69%. There was a significantly higher number of IL-5$^+$ submucosal cells in control subjects compared with numbers seen in children with STRA (median: STRA, 47.3/mm^2 [IQR, 27.6-96.4/mm^2] vs control subjects, 154.3/mm^2 [IQR, 72.6-257.1/mm^2]; P <.05; Fig 3, A). There were no significant differences in numbers of submucosal IL-13$^+$ cells between patients with STRA and control subjects (median, 15.95/mm^2 [IQR, 9.52-34.2/mm^2] vs 8.58/mm^2 [IQR, 2.08-102.3/mm^2]), respectively (Fig 3, B).

Relationship between IL-5 levels and eosinophilic inflammation

There was no difference in biopsy specimen, BAL fluid, or sputum eosinophil counts in subjects with STRA in whom IL-5 was detected compared with counts in those in whom IL-5 was undetectable (see Fig E3). Those children in whom IL-5 was detectable in sputum had a significantly lower FEV_1 than those with absent IL-5 ($P < .05$).

Airway remodeling

Children with STRA had increased RBM thickness compared with that seen in control subjects (7.12 μm [IQR, 6.37-7.89 μm] vs 4.89 μm [IQR, 4.16-6.16 μm], respectively; $P < .0001$; Fig 4, A). Epithelial shedding was similar in both groups (Fig 4, *B*). Vv was significantly increased in patients with STRA compared with control subjects (0.20 [IQR, 0-0.65] vs 0.09 [IQR, 0-0.16]; P < .001; Fig 4, *C*). The subepithelial volume fraction of ASM indexed to the surface area of RBM (Vs) was also increased in patients with STRA compared with that seen in control subjects (19.83 [IQR, 10.85-36.93] vs 9.14 [IQR, 4.55-15.88]; $P < .01$;Fig 4, D).

Influence of atopy on inflammation, remodeling, and T_H2 cytokines

When all subjects were compared, BAL fluid and biopsy eosinophil counts were higher in atopic compared with nonatopic subjects. RBM thickness, epithelial shedding, and ASM Vv were also greater in atopic compared with nonatopic children (Tables IV and V). However,

atopic children with STRA had increased ASM mass compared with atopic control subjects (Tables IV and V).

Influence of steroids on inflammation, remodeling, and T_H2 cytokines

There was no relationship between dose of inhaled or maintenance oral steroids and any parameters of inflammation or detection of T_H2 cytokines in children with STRA (see Tables E6 and E7 in this article's Online Repository at www.jacionline.org). However, there was a negative association between ASM mass and oral steroid dose (ASM Vv: $r = -0.63$, $P < .002$; ASM Vs: $r = -0.73$, $P < .001$).

DISCUSSION

In this large pediatric bronchoscopic study, an important novel facet is the use of prior detailed evaluation to exclude as far as possible all those who were nonadherent or had other confounding issues. We have shown that, unlike in adults,[28-30] STRA in children is characterized by eosinophilic and not neutrophilic airway inflammation, there is no mast cell myositis,[31] and the eosinophilic inflammation is not driven by signature T_H2 cytokines in most patients.[9]

Quantification of BAL fluid cytokines was performed by using 2 separate assays, and levels of T_H2 cytokines remained undetectable in patients with STRA and control subjects by using both techniques, despite the limits of detection for T_H2 cytokines with both assays being low. Indeed, the assays were sensitive enough to detect the levels of cytokines found in studies of severe eosinophilic asthma, with a comparable degree of airway eosinophilia.[32] In addition, induced sputum supernatants were analyzed because these are less dilute with similar results. However, our sputum samples were processed with DTT, which can affect cytokine measurements.[33] Inspection of the calibration curves (see Fig E2 in this article's Online Repository at www.jacionline.org) showed that DTT did not affect IL-5 quantification but might have reduced the sensitivity for IL-4 and IL-13. DTT rendered sputum eotaxin completely undetectable. Finally, to support the BAL fluid and sputum findings, submucosal IL-5 and IL-13 expression were also quantified in biopsy specimens. Again, no differences were found in the expression of IL-13 between patients with STRA and control subjects, and if anything, IL-5^+ cell counts were higher in control subjects. However, the spread of data is wide (Fig 3, *A*) and suggests the control subjects likely represent the normal range (because they include atopic subjects). We acknowledge that our control subjects were having a clinically indicated bronchoscopy and were therefore undergoing invasive procedures for significant medical problems, which might be a confounder. However, cytokine levels were predominantly undetectable in both groups, suggesting this confounder did not cause increases in false-positive results. Eight (15%) of 53 children with STRA had detectable levels of BAL fluid and sputum IL-5; however, even in these children, there was no relationship between sputum, BAL fluid, or biopsy eosinophil counts and IL-5 levels. We therefore believe that failure to detect increased T_H2 cytokine levels in our patients with STRA is real because only a very small number of children with STRA had any detectable levels in any compartment that we examined. Eight of 20 patients with STRA with BAL fluid and sputum samples had no detectable T_H2 cytokines in either. None of the patients with detectable T_H2 cytokines in sputum and BAL fluid had high tissue cytokine expression (defined as greater than the mean value for control subjects in biopsy specimens). Even levels of IL-5, which could be reliably quantified in BAL fluid, sputum, and biopsy specimens and is most closely related to eosinophil recruitment, were not increased.

When we split the children with STRA into those with any detectable BAL fluid T_H2 cytokines (IL-4, IL-5, or IL-13) and undetectable T_H2 cytokines, the former had

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significantly lower spirometric results and greater BDR (Table III), suggesting more severe disease. Looking for the presence of any T_H2 cytokines in children with STRA might therefore be a biomarker that allows identification of the subgroup more likely to respond to specific antibody therapies directed against T_H2 cytokines. This suggests that pediatric STRA is heterogeneous and that treatment needs to be individualized. However, when children with STRA were split according to clinical criteria into those with persistent symptoms or those with frequent exacerbations, there was no difference in T_H2 cytokine levels between the 2 groups.

Another notable finding is the wide variation in BAL fluid and biopsy eosinophil counts between subjects. Although eosinophil counts were higher in children with STRA as a group compared with control subjects, within the group, eosinophil numbers were very different. This finding further underscores that STRA in children is a heterogeneous disease.

It is likely that T_H2 cytokines were undetectable because these children were receiving high-dose maintenance steroids. This might not have been found in previous studies because of a failure to address adherence adequately. Importantly, the samples were taken when the children were receiving their usual prescribed medication but before a high-dose systemic steroid trial, which, if given before the bronchoscopy, might otherwise have modified the airway pathology. Although some subjects were prescribed low-dose maintenance oral steroids at baseline, *post hoc* analysis showed no difference between the children who were and were not prescribed oral steroids and no relationships between inhaled or oral steroid dose and airway pathology. We cannot exclude the possibility that T_H2 cytokines had initiated eosinophilic inflammation in patients with STRA, but we have presented clear evidence that these cytokines do not propagate established eosinophilic inflammation. We believe our data suggest that the classical T_H2 cytokines IL-4, IL-5, and IL-13 are steroid sensitive and therefore undetectable and that other steroid-resistant mediators are likely responsible for driving eosinophilic inflammation in patients with severe therapy-resistant disease. Although IL-17 might be a candidate,[34] the absence of neutrophilic inflammation and the fact that the source or role of IL-17[35] in this group is not known mean mechanisms need to be explored in experimental models[36] before being confirmed in pediatric airway samples because only limited samples (both size and volume) can be obtained from children.

Our data have important practical implications. The anti–IL-5 mAb mepolizumab has been shown to be beneficial in adults with persistent airway eosinophilia and multiple asthma exacerbations.[37-39] Our findings suggest that there is little indication for anti-T_H2 mAb therapy in many children with STRA. In children, at least, the presence of sputum eosinophilia should not be uncritically assumed to be synonymous with T_H2-driven inflammation. There is a clear need to elicit the mechanism of this apparently non–T_H2-mediated airway eosinophilia to develop appropriate biological therapies in children with severe disease.

This study includes the largest cohort of children with genuine STRA that has been investigated invasively before a steroid trial. Previous pediatric reports have only included patients with difficult asthma and inflammatory data after a steroid trial.[1,13] This is crucial because there is no reason to suppose *a priori* that airway pathology will be the same in a patients with steroid-resistant asthma as in a child who is nonadherent to ICSs.

There are a number of limitations. The study is cross-sectional, but this is inevitable because longitudinal invasive studies are unethical in children. Subjects with milder disease undergo bronchoscopy rarely, and therefore the data are only applicable to children with STRA. However, a previous study in children with mild-to-moderate asthma has shown increased tissue expression of IL-4 and IL-5,[40] which is in contrast to our findings. Also, because

control subjects were age matched and isolated upper airway problems are less common in school-aged children than those less than 5 years old, recruitment was difficult, and thus numbers were small.

Atopy was not the main focus of the study because so few of the children with STRA were nonatopic. However, increases in biopsy specimen and BAL fluid eosinophil counts, epithelial shedding, RBM thickness, and ASM mass were present in atopic compared with the very few nonatopic subjects, regardless of disease status. These findings are in contrast to a previous report in children with mild-to-moderate asthma, which showed similar pathologic changes in atopic and nonatopic subjects.[40] It is possible that there is an increased influence of atopy on pathology as disease severity increases. Importantly, the previous report did not include atopic nonasthmatic subjects, whereas 7 of 15 of our control subjects were atopic. When these subjects were compared with atopic patients with STRA, we found increased BAL fluid eosinophil counts, RBM thickness, and ASM mass in asthmatic patients.

In summary, we have shown that children with STRA have airway eosinophilia to varying degrees without neutrophilia or increased mast cell counts. There is considerable between-patient variability within this group. Importantly, signature T_H2 cytokines were absent in the majority of children. This report high-lights that there might be different mechanisms driving pediatric as opposed to adult STRA and carries the implication that successful adult therapeutic strategies should not be uncritically extrapolated to children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ASM	Airway smooth muscle
BAL	Bronchoalveolar lavage
BDR	Bronchodilator reversibility
CV	Coefficient of variation
DTT	Dithiothreitol
H&E	Hematoxylin and eosin
ICS	Inhaled corticosteroid
IQR	Interquartile range
RBM	Reticular basement membrane

STRA	Severe therapy-resistant asthma
Vs	Subepithelial volume fraction of airway smooth muscle indexed to surface area of RBM
Vv	Subepithelial volume fraction of airway smooth muscle indexed to subepithelial tissue

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Key messages

- Pediatric STRA is characterized by airway eosinophilia and remodeling but an absence of the classical T_H2 cytokines thought to drive allergic asthma.
- There was no neutrophilia or increase in mast cell inflammation in children with STRA, unlike in adults.
- A small subgroup of children with STRA had detectable T_H2 cytokines, suggesting phenotypic heterogeneity in this group.
- Treatments, such as anti–IL-5 antibody, might be ineffective in many children with STRA, and alternative mediators driving the disease need to be sought.

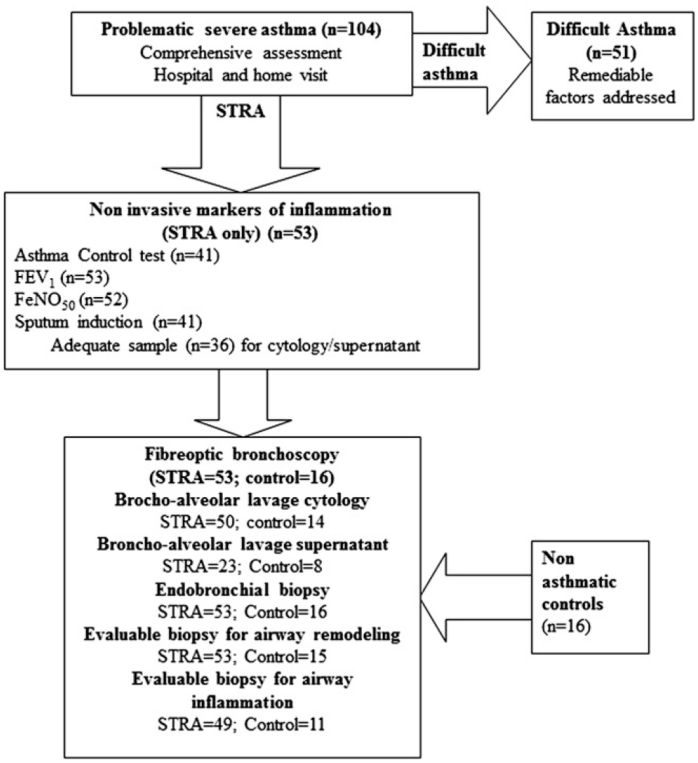

FIG 1.

Flow diagram illustrating the number of children with problematic severe asthma who were assessed and how many patients with STRA underwent invasive investigations to assess airway pathology. F_{ENO50}, Fraction of exhaled nitric oxide at 50 mL/s.

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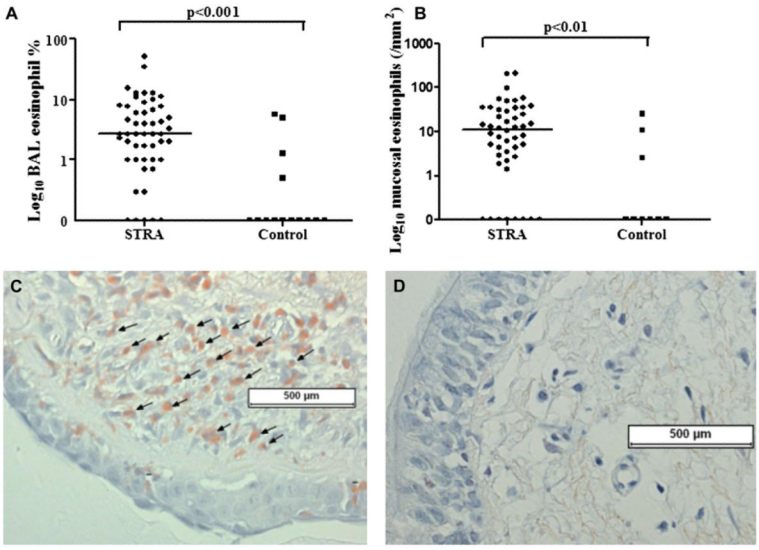

FIG 2.

A and **B**, Increased BAL fluid eosinophil (Fig 2, *A*) and submucosal eosinophil (Fig 2, *B*) counts in children with STRA compared with those seen in control subjects. **C**, A 5-μm paraffin section stained with Congo red dye showing positively stained eosinophils (*black arrows*) in the submucosa of a patient with STRA compared with an absence of submucosal eosinophils in a control subject (**D**; original magnification ×400).

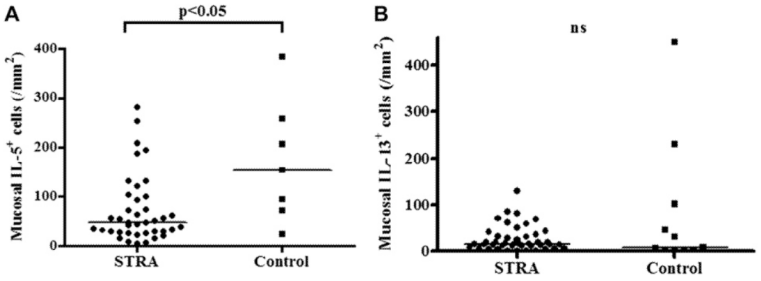

FIG 3.

Submucosal IL-5$^+$ (**A**) and IL-13$^+$ (**B**) cell counts in biopsy specimens from children with STRA compared with control subjects. Submucosal IL-5$^+$ cell counts were higher in control subjects compared with those seen in patients with STRA, and levels of IL-13$^+$ cells were similar in both groups. Analyses were performed with the Mann-Whitney *U* test. *ns*, Not significant.

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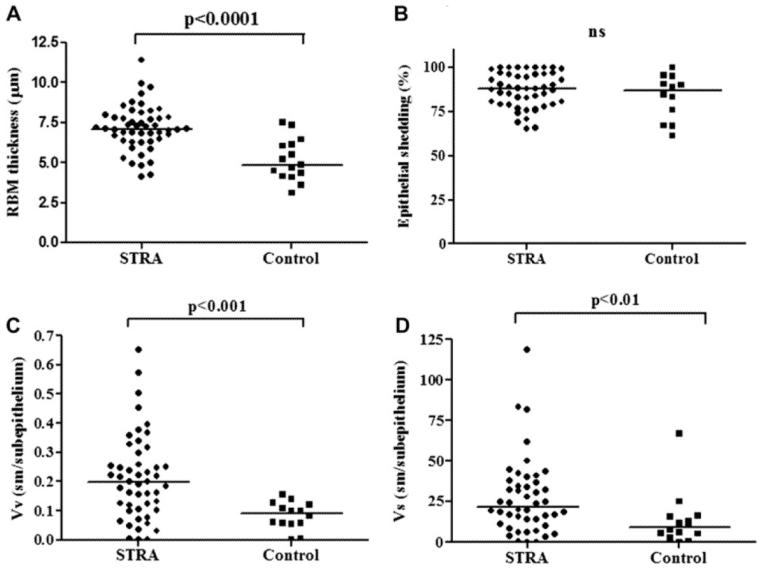

FIG 4.

Markers of airway remodeling showing increased RBM thickness in patients with STRA compared with control subjects (**A**), similar epithelial shedding in patients with STRA and control subjects (**B**), increased volume of ASM indexed to subepithelium in patients with STRA compared with control subjects (**C**), and increased volume of ASM indexed to RBM in patients with STRA compared with control subjects (**D**). *ns*, Not significant.

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TABLE I

Clinical characteristics of children with STRA and nonasthmatic control subjects

	Children with STRA (n = 53)	Control subjects (n = 16)
Age (y)	11.97 (9.97-14.4)	10.65 (7.54-12.96)
Male sex	33/53 (62%)	8/16 (50%)
Atopy	45/53 (85%)	7/15 (47%) *
Age at first symptoms (y)	1 (0.25-2.25)	—
Duration of symptoms (y)	10.1 (8.3-12.7)	—
Previous intubation for asthma	11/53 (21%)	—
Parental smoking	13/53 (25%)	2/9 (22%)
Percent predicted FEV_1	68.5 (54.8-86.5)	94 (85-106) †
BDR (%)	15.6 (5.5-23.4)	2.5 (0-8.5)
Persistent airflow limitation	13/53 (25%)	—
Total IgE (IU/mL)	386 (115-1286)	40 (14-130)
Medications		
ICS (mg/d) ‡	1.6 (0.8-1.6)	—
Maintenance oral prednisolone	24/53 (45%)	—
Long-acting β-agonist	53/53 (100%)	—
Leukotriene receptor antagonist	31/53 (58%)	—
Theophylline	12/53 (23%)	—
ACT score (/25)	13 (9-17)	—
$FENO_{50}$ (ppb; normal, <24 ppb)	50.3 (29.3-69.7)	—
Sputum eosinophils (%; normal, <2.5%)	7.5 (3.2-30.4)	—

Values are presented as medians (IQRs).

ACT, Asthma Control Test; *FENO*50, fraction of exhaled nitric oxide at a flow rate of 50 mL/s.

* $P < .01$.

† $P < .05$.

‡ Budesonide equivalent dose.

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TABLE II

Airway inflammation in children with STRA compared with nonasthmatic control subjects

Cell count	Patients with STRA (n = 53)	Control subjects (n = 16)	P value
BAL fluid neutrophils (%)	3.5 (2-7.2)	2.7 (1.2-7.5)	.37
Biopsy specimen neutrophils (/mm^2)	8.3 (0-26.4)	1.2 (0-20.0)	.32
Biopsy specimen mast cells (/mm^2)	45.7 (25.2-69.6)	60.5 (45.1-76.4)	.20
Biopsy specimen CD45$^+$ cells (/mm^2)	149 (3.7-419.9)	209.7 (168.8-244)	.11
Biopsy specimen CD8$^+$ cells (/mm^2)	51 (17.9-80.5)	54.8 (10.1-116.8)	.60
Biopsy specimen CD4$^+$ cells (/mm^2)	145.4 (77.0-224.5)	176.5 (23.5-259)	.92
Biopsy specimen CD4/CD8 ratio	3.30 (1.77-5.89)	2.97 (1.86-12.43)	.93

Values are presented as medians (IQRs).

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TABLE III

Clinical characteristics of children with STRA with any detectable BAL fluid T_H2 cytokines (IL-4, IL-5, or IL-13) compared with those without detectable T_H2 cytokines

	Detectable BAL T_H2 cytokines (n = 15)	**No detectable BAL T_H2 cytokines (n = 8)**	***P* value**
Age (y)	11.2 (9.4-14.4)	11.7 (10.0-12.3)	.78
Steroid dose:			
Maintenance (mg/d)	6.25 (5.0-7.5)	5.6 (5.0-7.2)	.80
ICS	1.6 (1.2-1.6)	1.4 (0.9-1.6)	.27
Exacerbations (in past year)	8 (4-8.5)	5 (4-10)	1.0
Admissions in past year	1.5 (0-5)	0 (0-10)	.70
Percent predicted FEV_1	**65 (42.5-71)**	**91.5 (83.5-96.8)**	**<.001**
Percent predicted FVC	**90 (61-99)**	**106 (94.5-110)**	**.03**
BDR (%)	**19 (14-33)**	**8.1 (3.6-17.4)**	**.03**
ACT score (/25)	10 (8-17)	13.5 (8-16.5)	.47
BMI (kg/m^2)	**17.6 (16.1-18.6)**	**23.8 (21-28)**	**.009**
BMI percentile	**66 (22-78)**	**99 (96-99.7)**	**.005**

Values are presented as medians (IQRs). Boldface text indicates statistically significant results.

ACT, Asthma Control Test; *BMI*, body mass index; *FVC*, forced vital capacity.

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TABLE IV

Differences in airway inflammation and remodeling between atopic and nonatopic subjects

	All atopy (n = 52)	All without atopy (n = 16)	*P* value	STRA with atopy (n = 45)	STRA without atopy (n = 8)	*P* value	Control with atopy (n = 7)	Control without atopy (n = 8)	*P* value
Percent predicted FEV_1	**68.5 (58.3-85.5)**	**86 (81-96)**	**.04**	66 (55.8-83.1)	81.5 (52.3-92.3)	.53	94.8 (79.4-107.8)	92 (86-105)	.78
BDR (%)	14 (5.3-22.7)	16 (11-28.9)	.94	15.6 (6.9-23.2)	16 (1.1-28.9)	.78	2.5 (0-8.5)	*	*
$Feno_{50}$ (ppb)				56.5 (31.4-70.6)	40.8 (15.3-49.5)	.11			
Serum IgE (IU/mL)	**428 (150-1354)**	**28.5 (11.3-117)**	**<.001**	**440 (202-1452)**	**74.5 (16.8-166.7)**	**.001**	97 (33-1101)	23 (4-43)	.07
Blood eosinophils (%)	**4.1 (1-9)**	**1.4 (0-2)**	**.05**	4.2 (0.95-9.2)	1.3 (0-6.7)	.29	**3.1 (2.2-5.9)**	**1.5 (0.7-2)**	**.052**
Sputum eosinophils (%)				7.5 (3.2-30.4)	9.4 (3.1-18)	.86			
Sputum neutrophils (%)				33 (12.5-71.1)	51.5 (24.1-74.0)	.42			
BAL fluid eosinophils (%)	**2.7 (1-6)**	**0 (0-4)**	**.01**	2.7 (1-7)	3.4 (0.3-8.8)	.83	0.9 (0-5.2)	0 (0)	.07
BAL fluid neutrophils (%)	3.3 (1.3-8.0)	3.7 (2-6.7)	.90	3.3 (1.2-7.3)	4.5 (3.4-8.8)	.43	4 (1.2-10.7)	2 (1-3.7)	.44
Biopsy specimen eosinophils (/mm^2)	**12 (2.6-34.7)**	**0 (0-8.3)**	**.01**	12.9 (3.3-35.3)	4.13 (0-10.98)	.07	0 (0-17.9)	0 (0)	.79
Biopsy specimen neutrophils (/mm^2)	7.64 (0-25.5)	0.8 (0-28.8)	.50	9.61 (0-25.7)	3.1 (0-40.3)	.94	2.4 (0-30.9)	0 (0-17.8)	.56
Biopsy specimen mast cells (/mm^2)	46.5 (29.2-69.9)	55.8 (23.4-72.6)	.79	45.8 (29.2-68.3)	40.2 (17.2-87.4)	.70	62.7 (20.2-117.3)	63.7 (50.5-73.7)	1.0
Biopsy specimens $CD4^+$ cells (/mm^2)	151.6 (92.3-225.4)	124.9 (24.2-239.1)	.49	151.6 (108-225.4)	74.5 (21.8-355.2)	.20	116.2 (23.5-286.1)	198.8 (89.01-249.1)	1.0
Biopsy specimen $CD8^+$ cells (/mm^2)	31.0 (14.9-84.3)	60.4 (23.6-84.4)	.54	51 (16.8-84.3)	58.1 (21.6-84.8)	.77	30.8 (4.7-115.1)	60.4 (32.9-88.9)	.49
Biopsy specimen $CD45^+$ cells (/mm^2)	164.4 (88.3-205.4)	201.1 (134.3-282.7)	.07	156.6 (88.3-202.4)	160.7 (107.1-373.2)	.40	190.9 (54.1-248.2)	228.9 (197.4-244)	.69

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	All atopy (n = 52)	All without atopy (n = 16)	*P* value	STRA with atopy (n = 45)	STRA without atopy (n = 8)	*P* value	Control with atopy (n = 7)	Control without atopy (n = 8)	*P* value
Biopsy specimen CD4/CD8 ratio	3.47 (1.8-5.5)	2.4 (0.3-12.0)	.59	3.47 (1.8-5.5)	2.1 (0.29-25.9)	.54	2.9 (2-9.4)	3.3 (1.3-9.7)	.89
RBM thickness (μm)	**7.10 (6.30-7.84)**	**4.83 (4.22-6.69)**	**.001**	**7.26 (6.79-8.13)**	**6.29 (4.96-7.23)**	**.04**	6.1 (4.9-6.5)	4.35 (3.63-4.71)	.07
Epithelial shedding (%)	**88.8 (79.0-96.3)**	**81.9 (66.3-89.8)**	**.04**	88.6 (79.8-96.5)	79.9 (68.3-87.3)	.06	90.1 (71.3-95.2)	83.9 (64.2-95.1)	.79
ASM Vv	**0.18 (0.10-0.26)**	**0.1 (0.05-0.16)**	**.02**	0.2 (0.1-0.35)	0.16 (0.10-0.25)	.52	0.10 (0.06-0.14)	0.06 (0.005-0.10)	.10
ASM Vs	**19.8 (10.5-36.1)**	**8 (4-20.4)**	**.03**	21.8 (10.9-37.4)	20.4 (11.9-39.1)	.87	**14.7 (9.0-35.5)**	**5.4 (0.60-8.0)**	**.04**

Values are presented as medians (IQRs). Boldface text indicates statistically significant results.

Feno$_{50}$, Fraction of exhaled nitric oxide at a flow rate of 50 mL/s.

* There were no patients in this group who had this investigation done, and therefore a *P* value could not be calculated.

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TABLE V

Differences in TH2 cytokine levels between atopic and nonatopic subjects

	All atopy (n = 52)	All without atopy (n = 16)	*P* value	STRA with atopy (n = 45)	STRA without atopy (n = 8)	*P* value	Control with atopy (n = 7)	Control without atopy (n = 8)	*P* value
BAL fluid IL-4 (pg/mL)	U (U-0.52)	U (U-0.5)	.74	0.23 (0-0.56)	U (U-0.39)	.37	U	0.3 (U-0.69)	.49
BAL fluid IL-5 (pg/mL)	U (U-1.39)	U	.49	U (U-1.42)	U (U-1.1)	.79	U	U	1.0
Biopsy specimen IL-5 (/mm^2)	55.7 (26.7-132.7)	42.7 (31.7-75.2)	.70	51.8 (26.3-107.5)	36.1 (28.3-49.3)	.40	206.2 (48.1-320.6)	124.7 *	*
BAL specimen IL-13 (pg/mL)	U (U-4.58)	U	.58	U (U-6.01)	U (U-16.7)	.97	U	U (U-17.0)	1.0
Biopsy specimen IL-13 (/mm^2)	16.0 (7.5-39.1)	12.2 (0-59.4)	.51	16.4 (11.7-33.7)	12.2 (0-59.4)	.40	6.0 (2.1-102.3)	19.8 (4.3-240.5)	.79

Values are presented as medians (IQRs).

U, Undetectable.

* There were only 2 patients in this group and therefore a *P* value and IQR could not be calculated.