

Induced sputum: Comparison of postinfectious cough with allergic asthma in children

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Background: Cough persisting after a respiratory infection is common in children and is often managed as asthma. However, little is known about the pathophysiologic mechanisms of such cough and how it compares with asthma.

Objective: We used the technique of induced sputum to examine the inflammatory index values associated with persistent cough or allergic asthma in children. We hypothesized that the sputum from children with persistent postinfectious cough would differ from that of children with allergic asthma in that the former would lack eosinophils compared with the latter.

Study design: Sputum production was induced with hypertonic saline solution in 34 children: 12 with cough persisting for 1 month or more after an apparent respiratory tract infection, not treated with corticosteroid; 11 with untreated atopic asthma, not using inhaled corticosteroid; and 11 with treated atopic asthma using inhaled corticosteroid.

Results: The percentage of eosinophils in the sputum of children with cough was significantly lower than in the sputum of children with untreated allergic asthma (median 0.5% vs 14.5%, $P < .0001$). Similarly, the percentage of eosinophils in the sputum of children with asthma treated with inhaled steroids was significantly lower compared with untreated asthmatic children (1.5% vs 14.5%, $P < .0001$). The peripheral blood eosinophils, serum eosinophil cationic protein, and nasal percent eosinophils of the patients with cough were also significantly lower than those from patients with untreated asthma. Methacholine challenge in 6 of the 11 cough patients tested showed mild-to-moderate hyperresponsiveness, whereas the other 5 had a negative methacholine challenge.

Conclusions: Children with persistent postinfectious cough do not have airway eosinophilia typical of untreated asthma. Despite the absence of eosinophilic inflammation, some of the patients with chronic cough had reactive airways. These results suggest that postinfectious cough in children has different pathophysiologic features than allergic asthma and probably represents a different disease. (*J Allergy Clin Immunol* 2000;105:495-9.)

Key words: Chronic cough, eosinophils, induced sputum, eosinophil cationic protein, IL-8, methacholine challenge, postviral

Abbreviations used

ECP: Eosinophil cationic protein

SST: Sodium sulfite titration

Intermittent and persistent cough are common in childhood¹ and are often triggered by viral infections or infections with atypical organisms.² Studies of children with chronic cough have found that approximately half have a positive methacholine challenge^{3,4} but that responsiveness to methacholine could not be predicted from the history.⁴ An epidemiologic study of children has suggested that recurrent cough in children has characteristics different from asthma and suggested that it should be considered a different disease.⁵ Studies in adults have attempted to determine the mechanisms of chronic cough and have recently applied the technique of induced sputum to detect and quantify airway eosinophilia.⁶⁻⁸ In children, less is known about the pathogenesis of chronic cough and how it compares with asthma. In the current study we used the technique of induced sputum to compare sputum cytologic features and chemistry in patients with postinfectious cough and those with allergic asthma, with and without inhaled corticosteroid treatment.

The technique of induced sputum is a minimally invasive technique that has been extensively studied and validated as a measure of airway inflammation.⁹⁻¹⁴ Sputum has been shown to yield measures of airway inflammation that correlate well with the more invasive bronchoalveolar lavage in asthma.¹³ The technique of induced sputum allows the safe examination of the inflammatory events in the airways of children with asthma or cough when bronchoalveolar lavage or biopsy of the airways would not be practical or ethical.¹⁴⁻¹⁹

We expected that sputum eosinophil counts in children with untreated allergic asthma would be higher than the eosinophil counts in sputum from children with allergic asthma treated with inhaled steroid. We hypothesized that sputum from patients with postinfectious cough would not be marked by eosinophils but might exhibit neutrophils. We also quantified eosinophils in nasal lavage and peripheral blood to assess systemic levels of eosinophils in these conditions and we measured eosinophil cationic protein (ECP) in sputum and serum. IL-8 was quantified in sputum as a chemokine associated with neutrophil chemotaxis.^{20,21}

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METHODS

Patient population

Patients with cough. Twelve consecutive patients meeting the inclusion criteria were recruited in the university-affiliated referral practice of one of the investigators. All children had the abrupt onset of paroxysmal cough associated with systemic symptoms of respiratory tract infection (fever, malaise, purulent nasal discharge, sore throat) that had subsided into a daily cough for more than 4 weeks by the time the patients were studied. Eight of the 12 had been treated briefly with inhaled steroid for a maximum of 2 weeks at the onset of the cough and had been off inhaled steroid at least 1 month before sputum induction. Patients were excluded if they had had episodes of symptomatic wheezing, responded to inhaled bronchodilator, or manifested other symptoms typical of asthma. Patients were also excluded if they had (1) a prior diagnosis of asthma, (2) an apparent viral respiratory tract infection within 2 weeks before entering the study or between entry and sputum induction, (3) been treated with inhaled steroids in the previous 4 weeks, (4) another definable cause for the cough (eg, postnasal drip, cystic fibrosis, or other systemic disease), (5) an abnormal chest radio-graph, or (6) episodic wheezing typical of asthma or bronchodilator responsiveness on spirometric testing. All patients studied demonstrated spontaneous resolution of the cough by 3 months after study.

Patients with asthma. Two groups of atopic patients with asthma were studied. All patients had positive skin prick tests to one or more common aeroallergens and exhibited bronchodilator responsiveness or a positive response to inhaled methacholine ($PC_{20} < 8$ mg/mL) as well as symptoms typical of asthma. The two groups consisted of 11 patients with minimally symptomatic asthma who were not being treated with inhaled steroid (untreated asthma) and 11 patients who were asymptomatic taking daily inhaled steroid at a constant dose for a minimum of 1 month (treated asthma) (6 were taking fluticasone propionate 250 µg/d and 5 were taking budesonide 400 µg/d). The study protocol was approved by the ethics committee of the Hospital For Sick Children, The University of Toronto. Informed consent was obtained from parents and assent from the patients.

Spirometry and methacholine PC_{20}

The procedures used followed established principles and procedures described previously.²²⁻²⁵ Flow volume curves were obtained with use of a Collins Eagle computerized system. The spirometer was calibrated daily with a 4-L calibration syringe before use. Bronchial responsiveness to methacholine was measured according to the method of Juniper et al.²⁶ The PC_{20} was calculated by interpolation of the last 2 data points on the noncumulative log concentration-response curve. One child in the postinfectious cough group was unable to perform the repeated forced expiratory maneuvers required for methacholine challenge although he produced a sample of sputum and performed adequate spirometry.

Sputum induction

Sputum was induced with hypertonic saline solution and prepared for cytologic examination by the method of Hargreave et al.⁶ Briefly, FEV_1 and forced vital capacity were measured before and 10 minutes after salbutamol inhalation (200 µg). The hypertonic saline solution was nebulized with a Medix ultrasonic nebulizer and inhaled for 7-minute periods for up to 21 minutes. After the measurement of FEV_1 , the concentration of saline solution was increased (after each 7-minute period) from 3% to 4% to 5%, provided the FEV_1 fell by $<10\%$ from the postbronchodilator value. A sputum sample was obtained after each concentration of saline solution and pooled.

Sputum processing

The method of sputum processing was as described by Pizzichini et al.²⁷ The sputum sample was transferred into a petri dish and its characteristics recorded in terms of color, consistency, and degree of contamination with saliva. The sputum plugs were selectively removed and transferred to a preweighed tube. The sample was then weighed and the volume recorded. A volume equivalent to 4 times the sample weight of a 1:10 dilution of Sputolysin reagent (dithiothreitol, Calbiochem-Novabiochem, San Diego, Calif) was added as a mucolytic agent. This was gently aspirated up and down with a Pasteur pipette, then vortexed to ensure adequate mixing, and placed on a bench rocker for 15 minutes. An equal volume of Dulbecco's PBS (Gibco BRL, Life Technologies, Grand Island, NY) was added to stop the action of the Sputolysin. This mixture was vortexed and then rocked for 5 additional minutes. After filtration through a 48-µm mesh filter, an aliquot was stained with trypan blue and a total cell count performed with a hemocytometer. A nonsquamous viability was then calculated. Cytospin preparations were prepared with the original filtrate, adjusted to equal approximately 1,000,000 cells per milliliter, and then stained with Wright-Giemsa stain. Differential cell counts were performed by counting 400 cells, which were identified as neutrophils, macrophages, eosinophils, lymphocytes, bronchial epithelial cells, and basophils. Remaining cell suspension was then centrifuged and aliquots of supernatant were frozen at -70°C for future analyses.

Blood and nasal samples

Blood for total eosinophils and serum ECP was drawn on the day of the sputum induction. Peripheral blood samples were collected into EDTA and sodium sulfite titration (SST) tubes (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ). The whole blood sample was used for the determination of the absolute eosinophil count with use of the Unopette Test (Becton-Dickinson) for eosinophil determination for manual methods. A minimum volume of 3.6 mm³ was counted to optimize accuracy. The SST tube was allowed to clot for 1 to 1.5 hours and then the serum was harvested and frozen in aliquots for later ECP determinations.

Nasal lavage was performed and the material handled as adapted from Koren et al.²⁸ ECP and IL-8 were measured in the sputum supernatant with use of commercially available kits (ECP, Pharmacia Upjohn, Toronto, and IL-8, Biotrak ELISA kit, Amersham International, Little Chalfont, UK).

Assessment of allergy

Patients were tested for allergy by skin prick test with a battery of 17 allergens including: *Alternaria*, *Cladosporium*, *Aspergillus*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, horse, tree pollen mixture, grass pollen mixture, ragweed, feathers, milk, egg, wheat, peanut, and soy (Bencard, Toronto, Ontario, Canada) and also with a negative diluent control and a positive histamine control. Wheal size was measured in 2 directions and a wheal of 3 mm in 1 direction was considered positive. Atopy was defined as 1 or more positive skin tests and the untreated asthmatic patients had to have a positive result to a pet animal in their homes.

Statistical analysis

Group data are expressed as the median and interquartile range (25th-75th percentiles) unless otherwise specified. In most of the comparisons across the 3 groups the variables were not normally distributed. For this reason the Wilcoxon rank sum test was used to test for differences between the groups. This nonparametric test does not require the standard parametric assumption that the variables be normally distributed in each group with equal variance.

Categorical binary variables including sex (male/female) were analyzed with the chi-square test. All statistical tests were 2 sided and exact values for the rank sum and chi-square statistic were calculated. Bonferroni's correction was used ($P = .05/3$) and only P values $<.017$ were considered to be positive. All analyses were performed with the SAS software package (SAS Institute, Cary, NC).

RESULTS

Patient demographics

The 12 patients with cough were significantly younger than the 22 asthma patients (cough, median age 9 years; asthma untreated, 13 years; asthma treated, 13 years, Table I) and only 4 of the 12 with cough were atopic. All the asthma patients were atopic. All patients had normal spirometry and there was no significant difference between the cough and asthma patients (Table I). The asthma patients had their asthma for several years, whereas the cough patients had persisting cough for a median of 3 months. Nine of the 12 cough patients had a prior history of coughing after most viral illnesses.

Induced sputum results

Patients with postinfectious cough had a median of 0.5% eosinophils in the sputum, which was significantly different from sputum from patients with untreated allergic asthma (Table II, 14.5%, $P < .0001$) but not significantly different from asthma patients treated with inhaled steroids (1.5%, $P = .032$). The 4 patients with cough who were also atopic did not have higher sputum eosinophil percentages than cough patients who were not atopic (data not shown). The absolute median sputum eosinophil counts were significantly lower in the cough patients than the untreated asthma patients (cough vs untreated asthma, $P = .0001$) but not significantly different than the treated asthma patients ($P = .05$) (data not shown). Sputum ECP levels from patients with cough were significantly lower than those from patients with untreated asthma but not treated asthma (Table II). The percent neutrophils in the sputum of cough patients (median of 45.9%) was not significantly different from the percent neutrophils in the sputum of untreated asthma (median of 22.0%, $P = .10$) or treated asthma (median of 36.5%, Table II). There were also no significant differences in the sputum IL-8 levels among the 3 patient groups (Table II).

The absence of significant eosinophilic inflammation in sputum from patients with postinfectious cough compared with asthma was paralleled by the significantly lower percent eosinophils in the nasal passages of patients with cough compared with asthma patients both untreated and treated (Table III, cough vs untreated asthma, $P = .0003$; cough vs treated asthma, $P = .001$). Similarly, the mean eosinophils in peripheral blood samples were lower in children with cough compared with both groups of asthmatics (Table III, cough vs untreated asthma, $P = .0001$; cough vs treated asthma, $P = .0001$). Serum ECP was significantly lower in patients with cough compared with both untreated asthma and treated asthma (Table III, cough vs untreated asthma, $P = .0008$; cough vs treated asthma, $P = .006$).

TABLE I. Patient demographics

	Cough	Asthma untreated	Asthma treated
No.	12	11	11
Age (y)*	9 (3.5)	13 (5)	13 (4)
Sex (female/male)	6/6	4/7	3/8
Atopic	4	11†	11
Duration	3 (1) mo‡	2 (3) y	7 (8) y
FEV ₁ (% predicted)	103 (20)	108 (15)	114 (22)

Values are median (interquartile range).

*Age (in years); cough versus untreated asthma, $P = .008$; cough versus treated asthma, $P = .002$.

†All sensitized to a pet in the home.

‡Nine of 12 had a prior history of persisting cough with respiratory tract infections.

Methacholine challenges

It was possible to test 11 of the 12 cough patients and 5 of them had a negative challenge whereas 6 subjects had a positive test (≤ 8). The median value for the entire group (assigning a value of 16 mg/mL to the negatives) was 2.55 mg/mL.

DISCUSSION

Sputum from children with persisting postinfectious cough differed from sputum of children with untreated allergic asthma (with significantly lower percents eosinophils and ECP in the sputum of the former compared with the latter). The patients with cough lacked evidence for eosinophil mobilization in the airways, nasal passages, and blood. They had no more eosinophils in the sputum than allergic asthma patients treated with inhaled steroid and were similar to published values for normal children.¹⁹ Thus children with cough persisting for 1 month or more after an apparent infectious illness have little evidence for involvement of eosinophils in the maintenance of the cough but some of them do have airway hyperreactivity as described previously.^{3,4}

Cough is a common complaint in children. In an epidemiologic study of children birth to age 5 years in the Toronto area, we found that about 10% had wheezing in the year before the study whereas a further 8% to 10% had episodes of cough.¹ Clinically this cough often occurs for a week to 2 weeks after viral respiratory tract infections, but in some instances the cough persists for 4 weeks or longer and then falls into the category of chronic cough.²

In 1975 McFadden²⁹ called attention to the fact that cough could be the sole manifestation of asthma. This was confirmed by Carrao et al³⁰ and later by other investigators in studies of children.³¹ The concept of cough-variant asthma led to persisting cough in children being treated with asthma medication, initially bronchodilators³² and now more commonly anti-inflammatory medication, particularly inhaled steroid.³³ Postinfectious cough occurs more commonly in children than in adults,² but the relationship of postinfectious coughing to cough-

TABLE II. Airway reactivity and induced sputum cellular profile: postinfectious cough versus allergic asthma, untreated and treated

	Cough	Asthma untreated	Asthma treated
PC ₂₀	2.55 (14.64)*	0.7 (2.2)	1.3 (3.1)
Sputum total cells†	647 (1,054)	482 (442)	1,191 (1,160)
Sputum (% eosinophils)	0.5 (0.5)‡	14.5 (19.3)	1.5 (2.8)
Sputum (% neutrophils)	45.9 (53.9)	22.0 (77.3)	36.5 (49.5)
Sputum (% macrophages)	36.6 (56.1)	31.0 (54.0)	51.3 (38.5)
Sputum (% lymphocytes)	3.4 (2.4)	4.0 (2.3)	3.3 (3.3)
Sputum ECP (μg/L)	200 (470)‡	757 (1,218)	513 (1,053)
Sputum IL-8 (pg/L)	6,171 (9,890)	4,932 (7,176)	8,921 (15,390)

Values are median (interquartile range).

*Of the 11 children with cough tested, 5 patients had a negative methacholine challenge and were assigned a value of 16 whereas 6 patients had a positive methacholine challenge (PC₂₀ <8 mg/L).

†Sputum total cells = $\times 10^3/\text{mL}$.

‡P values sputum percent eosinophils, cough versus untreated asthma, $P < .0001$; and untreated versus treated asthma, $P < .0001$. Sputum ECP, cough versus asthma untreated, $P = .010$. All other comparisons were not significant.

TABLE III. Eosinophil and serum ECP: comparison of peripheral blood and nasal lavage

	Cough	Asthma untreated	Asthma treated
Nasal (% eosinophils)	0.0 (1.5)*	26.5 (51.6)	16.1 (21.8)
Blood eosinophils ($10^9/\text{L}$)	0.15 (0.06)	0.56 (0.62)	0.45 (0.34)
Serum ECP	8.2 (2.3)	28.3 (18.3)	12.4 (12.0)

Values are median (interquartile range).

*P values: nasal percent eosinophils, cough versus untreated asthma, $P = .0003$; cough versus treated asthma, $P = .001$; blood eosinophils, cough versus untreated asthma, $P = .0001$; cough versus treated asthma, $P = .0001$; serum ECP, cough versus untreated asthma, $P = .0008$; cough versus treated asthma, $P = .006$; all other comparisons were not significant.

variant asthma and to more classic asthma remains poorly understood, with little understanding of differences in pathophysiologic mechanisms. A recent epidemiologic study of cough in children suggested that the clinical characteristics of these children were different from those of children with asthma and suggested that chronic or recurrent cough in children should be considered a different disease than asthma.⁵ This suggestion was also made as a result of a study of bronchoalveolar cell profiles in children with asthma or chronic cough where asthma was associated with eosinophils, whereas chronic cough, as in the current study, was not.³⁴

The current study shows that postinfectious cough in the children examined was not associated with eosinophilic inflammation but some children had airway hyperreactivity. This is in contrast to the subset of adult patients with chronic cough described by Gibson et al⁶ who had eosinophils in their sputum (eosinophilic bronchitis) but lacked airway hyperreactivity. Although the children with cough did not suffer clinically from wheezing or dyspnea, it is possible if not likely that airway narrowing occurred at some time during the acute episode. An epidemiologic study has examined the characteristics of children who coughed episodically with viral illnesses but never complained of wheeze.³⁵ During the acute episode there was a fall in peak flow readings but the

severity of their episodes was not influenced by the presence of atopy.

Numerous studies over the past decade with use of airway lavage or biopsy have established the presence of inflammation, particularly eosinophilic inflammation in the airways of adults with asthma, even mild stable asthma.^{36,37} Because of the invasive nature of these techniques, fewer studies have been done in children.^{34,38} In the current study allergic children with asthma who served as the positive controls were chosen from children who were sensitized to a pet in their home but were not receiving maintenance inhaled steroid. Such children were thought likely to have eosinophils in their sputum despite having only intermittent symptoms and normal spirometry because a similar group of children had been previously found to have elevated peripheral eosinophils and serum ECP that decreased after treatment with inhaled steroid despite the lack of symptoms.³⁹ In the current study a diary of symptoms was not kept before induction of sputum, but the spirometry was normal in all cases. As well as elevated sputum eosinophils, these children had elevated sputum ECP. The children did have airway reactivity on methacholine testing, fulfilling the criteria for asthma. The data suggest that allergic asthmatic patients with minimal symptoms can have elevated eosinophils and ECP in their sputum. The median sputum eosinophil percent of 14.5% was high for this group of mild asthmatic children. Children with mild asthma have been reported to have median eosinophil percentages less than 5%.¹⁹ The untreated allergic patients selected for this study were a group who were sensitized to a pet kept in their homes. The data suggest that such children, despite apparently mild asthma with minimal symptoms and normal spirometry, can have florid airway eosinophilia. These children might be at risk for remodeling of the airways and worsening of their asthma in the absence of treatment or elimination of the relevant allergens in their environment.

In contrast, the children with postinfectious persisting cough examined in the current study lacked any evidence

for eosinophilic inflammation in the airways, supporting the suggestion that cough in children is different from allergic asthma.^{5,34} The results lead to the question of appropriate treatment of children with postinfectious cough. There might be a difference in response to treatment with inhaled steroid depending on the presence or absence of eosinophils, much as has been described for adults.⁴⁰ The clinical management of such children should be studied separately from allergic asthma to determine the most appropriate treatment.

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