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Original Articles

Comparison of Atopic and Nonatopic Children With Chronic Cough: Bronchoalveolar Lavage Cell Profile

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Summary. Chronic cough is a common complaint in children and its relationship with asthma is controversial. The aim of the present study was to determine the pattern of airway inflammation in atopic and nonatopic children with chronic cough, and to investigate whether atopy is a predictive factor for eosinophilic inflammation in cough. Bronchoalveolar lavage (BAL; three aliquots of 1 ml/kg saline) was performed in the right middle lobe of 24 (11 atopic and 13 nonatopic) children with persistent cough (8 females, 16 males), mean age 4.7 years (range: 1–11). Atopy was defined as an elevated total serum IgE or a positive RAST test. Both atopic and nonatopic children with persistent cough had an increase in total cells/ml in BAL (atopic: median 39×10^4, range: 20–123; nonatopic: median 22×10^4, range: 17–132) compared to nonatopic controls (median 11×10^4, range 9–30). The increases were mainly in neutrophils (atopic: median 17%, range 2.5–88.5%; nonatopic: median 6%, range 1.0–55.0%) compared to controls (median 1.55%, range 0.5–7.0%; atopics vs. controls, $P < 0.005$). There were no significant increases in eosinophils, lymphocytes, epithelial cells, or mast cells. Eosinophils were elevated in only 5/11 atopic and none of the nonatopic children. The increased percentage of neutrophils in the BAL fluid of atopic and nonatopic children with persistent cough could be due to an underlying inflammatory process driving the cough, or even conceivably, due to the effect of coughing itself. In this highly selected series, the absence of eosinophilic inflammation in the majority suggests that most would be predicted not to respond to inhaled corticosteroid therapy. This study underscores the need to be cautious about treating coughing children with inhaled corticosteroids, even in the context of a tertiary referral practice. **Pediatr Pulmonol. 2007; 42:857–863.**

Key words: children; neutrophils; chronic cough; atopy; bronchoalveolar lavage; asthma; eosinophils.

INTRODUCTION

Cough is a primary defense mechanism that functions to protect the airways, clearing irritants and mucus. Cough can be classified into acute and chronic. Chronic cough can be defined as a cough persisting for at least 6 weeks[1] however this definition is arguable and others have regarded duration more than 4 weeks as chronic cough. Chronic cough is a common complaint in children, with a wide differential diagnosis. In a community setting, most

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children with chronic cough do not have asthma, although recent diagnostic fashion has been to assume that all chronic cough is due to asthma, and treat it with inhaled corticosteroids. Thus this common condition may frequently be overdiagnosed as asthma,[2,3] and harmful medication may be continued for a long period of time without benefit for these children. The relationship between chronic cough and asthma is controversial. Although cough variant asthma is recognized as a precursor of bronchial asthma,[4] it is not known whether chronic cough is also a precursor of asthma.[1]

Atopic patients with chronic cough due to cough variant asthma are thought to have airway inflammation similar to atopic patients with asthma, whose bronchoalveolar lavage (BAL) fluid contains eosinophils and mast cells.[5] In adults, eosinophils are believed to be a key effector cell in asthma. Some studies[6,7] have provided evidence of eosinophilic inflammation in the BAL of children with asthma. Marguet et al.[8] demonstrated that the BAL profile in asthma is clearly characterized by a high proportion of eosinophils, which is independent of the severity of the disease and treatment with inhaled steroids.

Most previous studies have studied milder forms of cough, and there is a paucity of data in a tertiary context, where one might expect there to be a greater likelihood of finding pathology.[9,10] The aim of the present study was therefore to determine the pattern of inflammation in atopic and nonatopic children with chronic cough, and to investigate whether atopy is a predictive factor for eosinophilic inflammation in this context.

METHODS

Patients

We studied 24 (11 atopic and 13 nonatopic) children (8 females and 16 males) with a mean age of 4.7 years (range 1–11 years) seen at the pediatric outpatient clinic of the Royal Brompton Hospital with a history of chronic cough (Table 1). All had coughed for at least 2 months. Fifteen had used inhaled corticosteroids, with no evidence of benefit. Fifty percent of these children had cough for periods longer than 1 year, and 21/24 (88%) have nonproductive (dry) cough. Inclusion criteria were chronic cough (cough for more than 6 weeks), absence of respiratory tract infection in the preceding 30 days, absence of alternative diagnosis such as cystic fibrosis, immunodeficiency, sinusitis and ciliary dyskinesia and agreement of parents after informed consent signature. Routine tests performed in these children included chest X-ray and high resolution CT scan, full blood count, serum immunoglobulins, sweat test, nasal brushing for ciliary motility and structure studies, 24-hr pH study. Spirometry and exhaled nitric oxide were performed in those older than 7 years old, and evaluation by an otorynolaryngologist performed when indicated.

Six children (three atopic and three nonatopic) were receiving inhaled steroids. Atopy was defined as a positive radioallergosorbent (RAST) test (eight children) or elevated total serum IgE (compared to age appropriate IgE ranges, three children). All children with chronic cough underwent bronchoscopy for clinical purposes. BAL was also performed on five children without chronic cough (control group) who underwent bronchoscopy for other clinical reasons, (three children underwent bronchoscopy for investigation of vascular and structural malformation and two for stridor). All were nonatopic and had no history of asthma.

None of the patients had a clinical history of respiratory infection in the 4 weeks prior to bronchoscopy, albeit 2/24 patients reported use of antibiotics for other reasons in this period (one received azythromycin and the other amoxicillin-clavulanate). Alternative diagnoses such as sinusitis, immunodeficiency, cystic fibrosis, tuberculosis, and primary ciliary dyskinesia were excluded in all patients by conventional tests. Chest radiographs showed opacities suggestive of atelectasis in only 4/24 children, but none of them had evidence of bronchiectasis on CT scanning.

Eighteen of the patients with chronic cough had also undergone a 24-hr esophageal pH test for gastroesophageal reflux (GER) as part of a separate study.[11] The test was defined as positive if the pH was less than 4 for greater than 4% of the 24-hr study period.

The study was approved by the Ethics Committee of the Royal Brompton Hospital, and written informed consent was obtained from the parents of the subjects, and age-appropriate assent from the children.

TABLE 1— Patient Characteristics

Variable	Controls	Nonatopic (range)	Atopic (range)
Gender	3F:2M	5F:8M	3F:8M
Median age (years)	9	4 (1–11)	6 (1–8)
Median of total serum IgE	—	21 (2–34)	101 (31–2,605)
Number of admissions in the last 12 months (median)	0	1 (0–4)	0.5 (0–5)
Inhaled steroids	0	3	3

F, female; M, male.

TABLE 2— Bronchoalveolar Lavage Cell Results

Variable	Controls	Nonatopic (range)	Atopic (range)
Gender	3F:2M	5F:8M	3F:8M
Median of BAL fluid recovery, %	70 (50.0–76.0)	50 (22.0–74.0)	50 (33.0–91.0)
Median of total cell count/ml $\times 10^4$	11 (9.0–30.0)	22 (17.0–132.0)	39 (20.0–123.0)
Median of differential BAL cell count			
Macrophages (%)	80 (73.0–87.0)	71 (35.0–82.0)	60 (11.0–88.0)
Lymphocytes (%)	12 (6.0–25.5)	11 (3.0–18.0)	7 (0.5–26.0)
Neutrophils (%)	1.50 (0.5–7.0)	6.0 (1.5–55.0)	17 (3.5–88.5)*
Eosinophils (%)	0.50 (0–1.50)	0.5 (0–2.0)	1.5 (0–10.5)
Mast cells (%)	0 (0)	0 (0)	0 (0)
Epithelial cells (%)	0 (0–6.50)	3 (0–23.0)	2.5 (0–4.0)

BAL, bronchoalveolar lavage; F, female; M, male.
*$P = 0.005$, atopic versus control group.

Bronchoalveolar Lavage

The same BAL protocol was used in all patients. The bronchoscopy was performed under general anesthesia using a laryngeal mask airway, or via a facemask with careful attention not to use suction until the bronchoscope had passed the vocal cords; the protected brush method was not utilized. BAL was performed in the right middle lobe in all patients and controls using three aliquots of 1 ml/kg saline (Table 2). BAL fluid was centrifuged at 1,500 rpm (300*g*) for 10 min at 4°C. The supernatant was removed and the cell pellet was resuspended in 2 ml of minimal essential medium (MEM) containing HEPES buffer (Invitrogen, London, UK). Total BAL cell counts were obtained using an improved Neubauer Counting Chamber and the results are expressed as number of cells per ml. Cytocentrifuge preparations were made using 100 µl aliquots of a 0.5×10^6 cells/ml suspension. After air-drying, the preparations were stained with May-Grünwald Giemsa to make differential cell counts. The percentages of each cell type were determined by counting 500 cells. A BAL sample was sent for microbiological analysis. Quantitative cultures were performed in selective media in the routine microbiological laboratory, and a bacterial count $\geq 10^5$ cfu/ml was considered positive. Additionally, direct immunofluorescence was performed in BAL samples using specific monoclonal antibodies against respiratory syncytial virus, influenza A and B, parainfluenza 1, 2, and 3, and adenovirus.

Statistical Analysis

The data were analyzed using nonparametric tests. The Mann–Whitney test was used to test differences between unpaired groups of quantitative data and differences were considered to be significant when $P \leq 0.05$.

RESULTS

BAL results are summarized in Table 2. Recovery of BAL fluid was similar in atopic (median: 50%, range: 33–91%) and nonatopic (median: 50%, range: 22–74%) patients, but lower than in the control group (median: 70%, range: 50–76%), albeit the difference was not statistically significant.

A nonsignificant increase in the number of total cells per ml of BAL fluid was observed in both atopic (median: 39×10^4, range: $20–123 \times 10^4$) and nonatopic (median: 22×10^4, range: $17–132 \times 10^4$) children with chronic cough when compared to controls (median: 11×10^4, range: $9–30 \times 10^4$). The increases in total cells were mainly due to increases in neutrophils in both the atopic (median: 17%, range: 2.5–88.5%) and nonatopic (median: 6%, range: 1–55%) children with chronic cough compared to controls (median: 1.5%, range: 0.5–7.0%). In total, 6 of the 13 nonatopic and 9 of the 11 atopic children with chronic cough had neutrophil percentage counts above the upper limit of 7% for the controls. However, the percentage of neutrophils was significantly higher only in the atopic children compared to controls, $P = 0.005$ (Table 2 and Fig. 1). Microbiological analysis

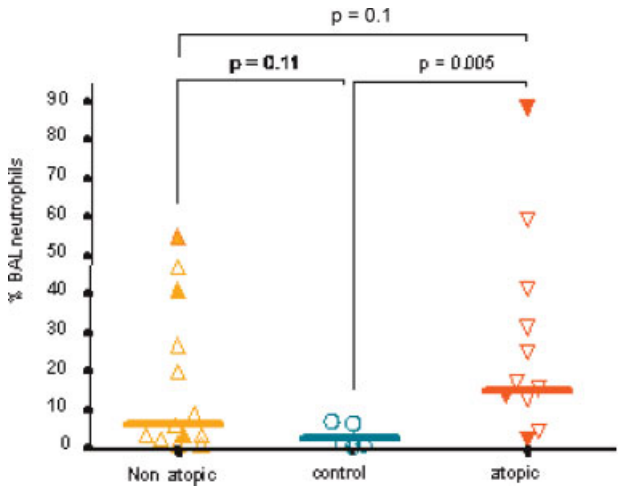

Fig. 1. Percentage of bronchoalveolar lavage (BAL) neutrophils in atopic, nonatopic and control children with chronic cough. Horizontal bars indicate the median for each group of patients (closed symbols = children using inhaled steroids).

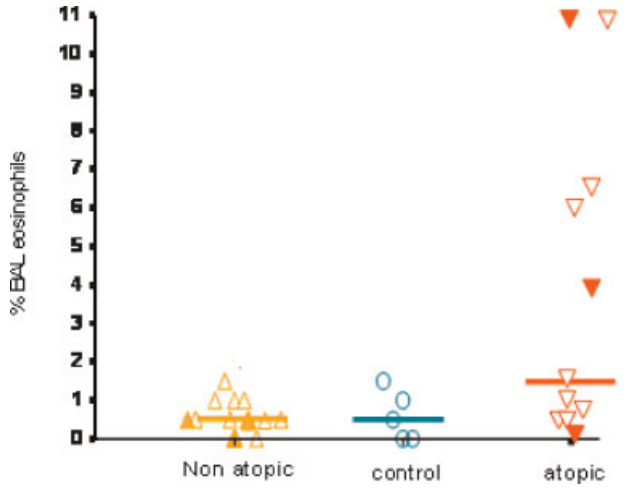

Fig. 2. Percentage of bronchoalveolar lavage (BAL) eosinophils in atopic, nonatopic and control children with chronic cough. Horizontal bars indicate the median for each group of patients (closed symbols = children using inhaled steroids).

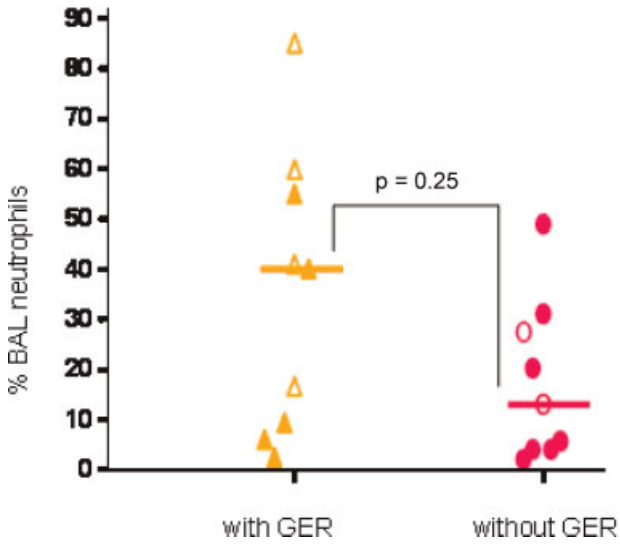

Fig. 3. Percentage of bronchoalveolar lavage (BAL) neutrophils in atopic and nonatopic children with and without gastro-esophageal reflux. Horizontal bars indicate the neutrophils median for each group of patients (closed symbols indicate non atopic children; open symbols indicate atopic children).

of the BAL samples was negative for bacteria and viruses in all cases.

There were no significant differences in the number or percentages of lymphocytes, epithelial cells or mast cells. The percentage of BAL eosinophils was elevated (>3%) in five of the 11 atopic children, although there was no increase in eosinophils in the nonatopic group (Fig. 2). However, the slightly higher percentages of eosinophils in the atopic group did not reach significance compared with the other two groups (Fig. 2).

Of the 18 children with chronic cough who underwent 24-hr pH testing for GER, nine were positive (four atopic and five nonatopic). Median BAL neutrophil percentages were higher in the nine patients with a positive GER (median: 41%, range: 1.5–88.5%) compared to the nine with a negative GER (median: 15.5%, range: 1–47%), but the difference was not statistically significant ($P = 0.25$, Fig. 3). Seven children with GER showed more than 10% of neutrophils in the BAL, although this finding was present in five children without GER. Lipid laden macrophages were measured in 23 of 24 children. Of the nine with a positive pH study, only three had an index greater than 100.

DISCUSSION

Chronic cough, defined as a cough persisting for at least 6 weeks, is a common symptom in childhood.[1] There is little information about the pathophysiology and the airway cellularity in children with chronic cough.[12–14] We analyzed the inflammatory cell profile of BAL fluid from atopic, nonatopic and normal children with chronic cough. The population of coughing children is highly selected, having been deemed sufficiently severe to warrant referral and investigation in a tertiary center; none had a diagnosis of a specific chronic chest disease such as bronchiectasis prior to diagnosis. The control children were matched for age as far as possible, but since ethically we could only perform a bronchoscopy in a child in whom it was clinically indicated, this group is necessarily limited. Nonetheless, our control data are similar to those of others who were able to study larger groups.[15]

In the present study, the majority of the atopic children and nearly half of the atopic children with chronic cough had an increase in the percentage of BAL neutrophils when compared to controls; but even in this selected group, eosinophilia was absent in the nonatopic coughers, and present in less than 50% even of atopic children with cough.

An important issue is the upper limit of normal of airway neutrophilia in children. Because healthy children cannot be studied for ethical reasons, pediatric BAL reference values are difficult to obtain. It is probable that the differential cytology in children is similar to that observed in healthy adults.[16] Although BAL neutrophil of 17% is the upper limit of normal reported by Middula et al.[17] (range 0–17%) and Ratjen et al.[18] (0–17%) in five other studies the median normal value for neutrophils is given as 3.5%[17–21] Hence in our study, the median value of BAL neutrophils (17%) and the values in both the non atopic group (3.5–88.5%) and atopic group (1.5–55%) were appreciably higher than the values reported in normal children.[15]

The mechanism of the neutrophilia is unclear. One of the most common causes of chronic cough in children is recurrent viral infection,[22] with some viral infections causing prolonged periods of cough. An increased

percentage of BAL neutrophils may be due to an underlying inflammatory process such as occult persistent infections, caused by bacteria or viruses.[13,23–25] In a recent cohort study published by Marchant et al.,[13] a standardized pathway of investigation of chronic cough in children revealed that protracted bacterial bronchitis diagnosed by BAL was the most common diagnosis among 108 children, with significant higher neutrophil levels on BAL samples.

None of our patients had clinical evidence of a respiratory infection in the 4 weeks prior to bronchoscopy, and microbiological analysis of the BAL samples was negative for bacteria and viruses. However, we cannot rule out the possibility of a viral infection in these children, because molecular biology methods were not utilized and may improve viral detection, while represent the main tool for detection of some significant respiratory viruses such as rhinovirus, coronavirus, and human metapneumovirus. Pertussis, another potential pathogen quoted as a substantial cause of prolonged cough in school age children[26] would be an unlikely etiology once 50% of our children had cough for periods longer than 1 year (range 1–10 years). However, we cannot exclude the possibility of a pertussis infection, because we did not do serology, and children who have been partial vaccinated may have prolonged dry cough without the other classical features of whooping cough.[27]

It is also possible that we have underdiagnosed other infections in this group. We defined a positive culture a $\geq 10^5$ colony forming units per milliliter of BALF because the bronchoscopy was performed using a laryngeal mask airway, or via a facemask with careful attention not to use suction until the bronchoscope had passed the vocal cords; the protected brush method was not utilized. The ERS task force defined this cut off for non protected BAL specimens.[15] Baker et al.[28] also recommended that the cut off level of 10^4 cfu/ml for BAL is appropriate when pneumonia is suspected and a cut off level of 10^5 cfu/ml for BAL is appropriate if the probability of disease is low. Lower cut off levels have indeed been used by some investigators[29] when a protected brush is employed ($>10^3$ cfu/ml) or with suspected ventilator-associated pneumonia ($>10^4$ cfu/ml). We acknowledge the difficulty of establishing a diagnostic threshold for quantitative culture on BAL for bronchitis in children[30] (as opposed to pneumonia). The second potential source of underdiagnosis is sampling error; although there are data on differences between BAL in the context of CF[31] we know of no such data on the chances of missing positive cultures in the context of bacterial bronchitis.

Another potential cause of chronic cough is GER. Irwin et al.[32] demonstrated that chronic cough can be the sole manifestation of GER in adults. Controversy exists regarding the presence of inflammatory markers in the BAL fluid of children with GER,[33] but some studies have shown that GER may increase the percentage of BAL neutrophils.[34] In the present study, 9 of 18 children (4 atopic and 5 nonatopic) had a positive 24-hr pH test (>4% of the study period with pH < 4). Seven children (four atopic and three nonatopic) who had a positive pH test also had an increased percentage of BAL neutrophils. Whether or not GER is associated with or is the cause of airway inflammation in children with chronic cough cannot be determined from these cross-sectional data (Fig. 3).

A higher percentage of BAL neutrophils in patients with chronic cough may also be explained by exposure to environmental tobacco.[35] Unfortunately, in the present study we did not actively investigate exposure of the children to tobacco smoke using urine or salivary cotinine. Conceivably, the mechanical effect of coughing could itself cause airway neutrophilia; to our knowledge, this has never been tested, and it is difficult to believe that such profound neutrophilia as reported here could be caused by the mechanical effects of cough alone.

Asthma is usually characterized by wheeze and dyspnoea, and there is controversy as to whether pure cough variant asthma exists.[36–40] Cough variant asthma is usually diagnosed in a child with persistent cough who has airway hyperresponsiveness and a good response to anti-asthma medication, with relapse of symptoms when the medication is stopped.[4] Atopic children with chronic cough due to cough variant asthma would be predicted to show airway inflammation similar to atopic children with asthma, whose BAL fluid contains eosinophils and mast cells.[5,41] Our results are consistent with the findings of other investigators, who found that chronic cough was rarely associated with eosinophilia in BAL or induced sputum.[8,13,42,43] Furthermore, data from the Tucson cohort study[44] have shown that cough without wheezing had a more favorable prognosis than cough with recurrent wheezing, suggesting that chronic cough differs from asthma in several aspects and may have a different pathophysiology. In the present study, atopy did not predict eosinophilic airway inflammation, suggesting that only a minority of these children have eosinophilic asthmatic-type airway inflammation.

A review[45] of the use of medications in children with persistent cough has demonstrated an overdiagnosis of asthma and overuse of asthma treatments, with a potential risk of side effects in children with chronic cough.[46,47] One Australian study[48] concluded that, although children with chronic cough were similar to asymptomatic children in terms of atopic status, family history and respiratory morbidity, the rates of asthma diagnosis and use of asthma medication was higher in these children.

In the present study, 6 (3 atopic and 3 nonatopic) of the 24 children were using inhaled steroids. We accept that it would have been more rigorous to stop this medication prior to bronchoscopy, but this was not thought to be

ethical. One child had an increase in BAL eosinophils, despite being prescribed inhaled steroids, although no increase in BAL eosinophils was observed in the others. It is possible that in these children inhaled corticosteroids might have suppressed BAL eosinophils. None of the patients reported any improvement in symptoms related to inhaled steroid therapy, and indeed, all were still symptomatic at the time of bronchoscopy despite this treatment. We do not have any information on adherence to therapy. However, as a group, children with chronic cough did not exhibit asthmatic-type airway inflammation, underscoring the need for caution in the diagnosis of cough variant asthma, even in patients with severe symptoms investigated in a tertiary center.

In conclusion, the evaluation of 24 children with persistent cough demonstrated that atopic and nonatopic children with chronic cough frequently have an increased percentage of BAL neutrophils, the cause of which is not known, but which may be due to an underlying inflammatory process. Further studies are needed to determine the mechanism of neutrophilia in children with chronic cough. Atopy did not predict eosinophilic airway inflammation in children with chronic cough. Few of the atopic children, and none of the nonatopics with persistent cough had asthmatic-type eosinophilic airway inflammation, and thus would be predicted not to respond to inhaled corticosteroid therapy.

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