Induced Sputum Cell Count and Cytokine Profile in Atopic and Non-Atopic Children With Asthma

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Summary. Background and aim: Sputum induction is a semi-invasive technique used to detect and monitor airway inflammation. In this study, the cell profile, and Th1 and Th2 cytokine levels in induced sputum of asthmatic and healthy children (HC) are compared. Methods: Sputum induction was performed in healthy and asthmatic children by inhalation of hypertonic saline solution. Differential cell count in the specimen obtained was carried out using optic microscopy. IFN-γ, IL-2, IL-10, IL-8, IL-6, IL-4, IL-5, IL-1β, TNF-α, and IL-12p70 levels were determined in sputum sample supernatants by flow cytometry. Results: Sputum induction was performed in 31 HC and 77 asthmatic children (60 atopic and 17 non-atopic asthma, NAA). Twenty-four samples were obtained in HC and 64 in patients. Median eosinophil count in atopic asthma (AA; 2%) was higher than in NAA ($P = 0.02$) or HC ($P = 0.01$). IL-4, IL-5, IFNγ, IL-2, and IL-12p70 concentrations were higher in AA than in NAA or HC. IL-8 was higher in asthmatic children (atopic and non-atopic) than in healthy ones. IL-10 was higher in the healthy group than in the AA group ($P = 0.02$). Conclusions: As compared to HC, the inflammatory profile in induced sputum of children with asthma showed an increase in proinflammatory cytokines. Concentrations of IL-10, an anti-inflammatory cytokine, were lower in children with AA than in HC. **Pediatr Pulmonol.** © 2013 Wiley Periodicals, Inc.

Key words: bronchial inflammation; asthma; allergy; children; induced sputum.

INTRODUCTION

Asthma is characterized by chronic airway inflammation, which manifests histologically as infiltration of the bronchial mucosa by inflammatory cells, epithelial cell shedding, mucosal cell hyperplasia, and submucosal thickening. In recent years, the study and monitoring of inflammation in asthma patients has acquired increasing importance as a complement to lung function testing and as a potential tool for prompt diagnosis of this disease.[1] Bronchial inflammation in asthma may be a direct reflection of the disease, whereas lung function study is a more indirect method for evaluating this condition. Induced sputum analysis, a semi-invasive technique that enables determination of the predominant cellularity and degree of inflammation in the samples obtained, has facilitated the study of inflammation in childhood asthma.[2–4] The number and types of cells and the inflammatory mediators present in patients with this condition differ from those found in healthy individuals.[5–8]

Several previous studies have investigated sputum cellularity in asthmatic children, and cell profile reference values have been described in healthy children (HC).[2,4] These studies usually compare a population of HC with pediatric asthma patients under treatment or not with inhaled corticosteroids and in different states of disease severity. Two studies have compared the

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induced sputum cell profile in children with atopic and non-atopic asthma (NAA).[9] The results of these studies suggest that the induced sputum cell pattern in non-atopic children is predominantly neutrophilic, while eosinophilia is the hallmark of airway inflammation in the majority of atopic wheezing children who are not receiving inhaled steroid treatment. Other studies have investigated the inflammatory phenotypes in acute asthma in children. Wang et al.[10] differentiated between eosinophilic and neutrophilic inflammation, whereas Gibson et al.[11] established three phenotypes in severe acute asthma (eosinophil exacerbation, eosinophil–neutrophil exacerbation, and non-eosinophil exacerbation). Some studies have demonstrated that cytokines are elevated in sputum of children with stable or acute asthma,[6,11–13] but none have established the various inflammatory patterns based on the cellularity and cytokine profile in sputum of atopic and NAA children. The objectives of our investigation were to study the atopic and non-atopic sputum cell profiles and patterns of cytokine production in children with asthma of varying severity in a stable phase, and to establish the normal induced sputum cell profile in HC in our setting. This is the first study to analyze, in particular, cytokine levels in induced sputum of atopic and non-atopic children with stable asthma.

MATERIALS AND METHODS

Study Population

This is a cross-sectional, observational, and descriptive study. The asthma sample included patients consulting in our outpatient pediatric pneumology clinic over a two-year period (2005–2006), seen on the one day of the week in which sputum induction and processing could be carried out. The sample of HC was comprised of children undergoing a yearly check-up in the general pediatrics clinic over another two-year period (2007–2008), and the sons and daughters of hospital staff. Written informed consent from parents and children (when applicable) was obtained for participation in the study and for each individual sputum collection. The study was approved by the Ethics Committee of Vall d'Hebron Research Institute.

Inclusion criteria: patient requirements were age between 7 and 15 years and a diagnosis of bronchial asthma, regardless of the severity. The condition had to be in a stable phase, defined as an absence of asthma symptoms (wheezing or difficulty breathing) and no need for short-acting beta2-agonists or oral corticosteroids during the previous month.

The diagnosis of asthma was based on consistent clinical symptoms, and a positive bronchodilator test according to GINA criteria.[14] Children with asthma were classified as atopic when they presented a positive skin prick test and/or elevated specific IgE levels (RAST technique) to any of the respiratory allergens tested. Inhaled corticosteroid use was allowed in up to 40% of the final cohort of asthmatic children. The HC (controls) had no personal history of asthma, rhinitis, or atopic dermatitis. They were also classified as atopic or non-atopic according to the same criteria.

Exclusion criteria: patients were excluded if they had other chronic respiratory diseases (e.g., cystic fibrosis, bronchiectasis, obliterating bronchiolitis, primary ciliary dyskinesia, interstitial lung disease), or had presented during the previous month an upper or lower respiratory tract infection or asthma symptoms, defined as wheezing or difficulty breathing and a need for short-acting beta2-agonists.

Study Variables

The following information was collected from a parental interview and the medical records: age, sex, atopy status, family background (asthma, atopy, rhinitis, or atopic dermatitis), bronchitis in infancy, smokers at home, age at the onset of asthma episodes (preceded or not by upper airway infection), number of episodes per year, symptoms between episodes, classification of asthma severity (infrequent episodic, frequent episodic, or persistent asthma),[15] treatment received, date of last episode, and date of last oral corticosteroid treatment.

The following determinations were performed in all patients: hemogram, total IgE, specific IgE antibodies (ImmunoCAP specific IgE; Phadia, Sweden) and skin prick testing (Leti allergen extracts; Leti Laboratories, S.L., Barcelona, Spain) to the following respiratory allergens: Dermatophagoides pteronyssinus, Dermatophagoides farinae, dog and cat dander, Alternaria spp., and pollen from olive, Parietaria, grasses, and plane tree (Platanus). Atopy was defined based on a specific IgE level of $\geq$0.35 kU/L or a positive skin prick test (wheals with a mean diameter of at least 3 mm were regarded as positive).[16]

The fraction of exhaled nitric oxide (FeNO) was determined by chemiluminescence (NIOX; Aerocrine, Sweden), following the recommendations of the American Thoracic Society (ATS) and European Respiratory Society (ERS).[17]

Sputum Induction

Sputum induction was performed with nebulized hypertonic saline solution (HSS), using a slight modification of the method described by Pizzichini et al.[18,19] Fifteen minutes before sputum induction, children underwent baseline spirometry testing and then inhaled 400 μg of salbutamol through a spacer/chamber. Children inhaled increasing concentrations of HSS (3%, 4%, and 5%) for 5 min per concentration, through a

mouthpiece and tube connected to an ultrasonic nebulizer (DeVilbiss Ultra-Neb 3000, Somerset, PA) that generated particles with a mean mass aerodynamic diameter of 4–5 μm. Nebulizer output was calibrated at 2.5 ml/min. FEV_1 was measured by forced spirometry after each 5-min period, and the test was interrupted when FEV_1 dropped by more than 15%. Salbutamol (500 μg) was added to hypertonic saline in patients with persistent asthma. Children rinsed their mouth with water before induction and between each 5-min period to minimize sample contamination with saliva. After each period, the child was encouraged to cough up sputum into a sterile container. A questionnaire was given to all children to determine the incidence and intensity of side effects related to sputum induction.

Sputum Examination

Sputum examination was performed as described by Pizzichini et al.[18] Sputum was separated from contaminating saliva and weighed. Samples had to weigh at least 50 mg to be accepted as valid. The aliquot was then dispersed with four volumes of freshly prepared dithiothreitol solution. The mixture was vortexed, and after being rocked for 15 min, four volumes of Dulbecco's PBS were added. The suspension was then filtered through 48-μm nylon gauze (Millipore Corporate Headquarters, Billerica, MA) and centrifuged at 2,500 rpm for 10 min. This resulted in the formation of a cell pellet and supernatant solution. The supernatant was stored at −80°C for analysis of inflammation factors. The proportion of salivary squamous cells was noted and cell viability was determined by the trypan blue exclusion method. The specimens were considered adequate if squamous cells represented less than 20% and viable cells 60% or more of the total cell population. Cytospin cell preparations were made using a cytocentrifuge. The cytospin slides were stained with May-Grünwald-Giemsa to determine the differential cell count, made by counting 400 cells per slide. Sputum eosinophilia was established when the percentage of eosinophils was ≥2.5%.[20]

Sample processing was performed by a person aware of the patients' condition. After processing, samples were frozen at −80°C and stored until analysis. Differential cell count and cytokine quantification were carried out by an investigator blinded to the origin of the samples.

Cytokine Measurement

IL-4, IL-5, IL-10, IL-6, IFN-γ, IL-2, IL-1β, IL-8, TNF-α, and IL-12p70 levels were determined in sputum sample supernatants by flow cytometry (Bender Medsystems, eBioscience, Inc., San Diego, CA). Because it has been reported that DTT may interfere with the detection of certain cytokines,[21] the cytokine standards were also treated with PBS–DTT dilution buffer to avoid bias in the results. The cytokine mix provided by the manufacturer was serially diluted to construct the standard curve. PBS–DTT dilution buffer without cytokines was used as a negative control. The limits of detection of the technique (pg/ml) were 20.8, 1.6, 1.9, 1.2, 1.6, 16.4, 4.2, 0.5, 3.2, and 1.5, respectively, for IL-4, IL-5, IL-10, IL-6, IFN-γ, IL-2, IL-1β, IL-8, TNF-α, and IL-12p70.

Data Analysis

Statistical analysis of the data was carried out with SPSS, version 15.0. The following parametric or non-parametric tests were applied depending on whether the groups followed a normal or non-normal distribution, and as appropriate: the Student *t*-test for unpaired data, one-way analysis of variance, the Mann–Whitney *U* and Kruskal–Wallis non-parametric tests, chi-squared test, Fisher exact test, and Pearson linear correlation. *P* values of <0.05 were considered significant. Normality was examined using the Kolmogorov–Smirnov test. Results are expressed as the median and interquartile range. A value of half the lower detection limit of the method was assigned to samples with undetectable cytokine levels to enable statistical comparisons.

RESULTS

Study Population

Three groups were studied: a control group of HC (n = 31), children with atopic asthma (AA) (n = 60), and children with NAA (n = 17). The three groups were comparable with regard to age, sex, weight, and height (Table 1). Of the 31 HC, 7 were atopic based on prick test or specific IgE results (none had rhinitis or atopic dermatitis). Asthmatic children of both groups presented a lower FEV_1/FVC ratio than the healthy controls, suggesting some degree of obstruction (Table 1). There were no differences in pulmonary function between asthmatic children treated with inhaled corticosteroids (ICS) (n = 27) and untreated children (n = 50). Some of the ICS-treated patients were also receiving other therapies: long-acting beta2-agonists (n = 24), montelukast (n = 15), tiotropium bromide (n = 4), azithromycin (n = 3), and oral corticosteroids (n = 3). The classification of asthma severity and FeNO values in the two patient groups, and the demographic characteristics and pulmonary function results in patients and controls are shown in Table 1. FeNO determination was not performed in HC.

Sputum Induction and Sample Quality

Adequate sputum samples (viability ≥60% and squamous cell contamination <20%) were obtained in 62%

TABLE 1— Demographic Characteristics and Baseline Pulmonary Function Results in Healthy Children, and Children With Atopic Asthma or Non-Atopic Asthma[1]

	Healthy children (n = 31), 24 NAHC, 7 AHC	Atopic asthma (n = 60)	Non-atopic asthma (n = 17)	*P* value
Age, y	12 (10–12)	11.5 (9–13)	11 (8.5–12.5)	0.60
Sex (% males)	55	68	65	0.21
Weight, kg	44 (37–51)	47 (34–59)	47 (35.5–59.5)	0.41
Height, cm	149 (139–160)	150 (138–164)	143 (138–161.5)	0.81
FVC, % predicted	93% (84–100)	92% (83–102)	88% (79.5–93.5)	0.19
FEV_1, % predicted	94% (101.5)	94% (94–102)	89% (62.5–98)	0.35
FEV_1/FVC, %	89.5% (87.2–93)	82% (78–87)*	85% (70.5–91.5)*	<0.001
FeNO, ppb		31 (15–55), n = 44	9 (6–15), n = 11	<0.001
Treated (T)		13 (7–34), n = 11	6, n = 3	0.04
Untreated (U)		34 (17–62), n = 33 (T vs. U, $P = 0.03$)	11 (9–16) n = 8 (T vs. U, $P = 0.03$)	<0.001
Asthma severity, n IE/FE/P		36/12/12	11/6/0	0.07
ICS treatment, n (%)		23 (38) IE 6, FE 5, P 12	4 (24) IE 0, FE 4, P 0	0.15
Drop FEV_1 5% HSS	2.2% (1.9–2.9)	5.3% (−1.5 to 14.2)	3.5% (1.1–9.4)	0.31

NAHC, nonatopic healthy children; AHC, atopic healthy children; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; FeNO, fraction of exhaled nitric oxide (in parts per billion); IE/FE/P, infrequent episodic, frequent episodic/persistent asthma; ICS, inhaled corticosteroids (160–1,200 μg budesonide equivalents); HSS, hypertonic saline solution.

[1]Results are expressed as the median (interquartile range). Other data are expressed as absolute or percent numbers.

*$P < 0.05$ when compared with controls.

of children (75% HC, 81% AA, and 58% NAA). The median cell viability of these samples was 76% in the HC group, 81% in AA, and 83% in NAA (Table 2).

Side effects of sputum induction were local and mild in all cases. As compared to controls (n = 31), asthmatic children (n = 77) more frequently presented cough (29% vs. 7%, $P = 0.01$), itchy throat (42% vs. 19%, $P = 0.03$), and sialorrhea (38% vs. 16%, $P = 0.03$). There were no differences between the groups for the remaining adverse effects studied (itchy eyes, unpleasant taste, and wheezing). Only five children, four in the AA group (two with infrequent episodic asthma and two with frequent episodic asthma) and one in the NAA group with frequent episodic asthma presented an FEV_1 drop >15% that reversed with inhaled salbutamol (400 μg).

Induced Sputum Cell Profile

The median (interquartile range) total cell count in induced sputum was 1.37×10^6 cells/g (0.54–3.1), 2.79×10^6 cells/g (1.49–5.56), and 4.49×10^6 cells/g (1.84–7.4) in HC, AA, and NAA, respectively, with significant differences only between HC and AA ($P = 0.02$).

Induced sputum total cell counts and cell profiles in the three groups are shown in Figures 1 and 2. Samples from children in the AA group showed a higher median percentage (range) of eosinophils (2% [2–9]) than those in the HC (0% [0–1]) or NAA groups (0% [0–0.25]; $P = 0.01$ and $P = 0.02$, respectively) and a higher median absolute number (0.02×10^6 cells/g [0–0.26]) than the HC group (0.0×10^6 cells/g [0–0.009]; $P = 0.001$); there were no differences between HC and NAA. Sputum eosinophilia was found in 45.5% of children with AA versus 5.5% (one child) of the controls and none of children with NAA ($P = 0.001$). There was no correlation between FeNO results and induced sputum eosinophils in AA (r = 0.29; $P = 0.09$) or NAA patients (r = 0.78; $P = 0.07$).

TABLE 2— Characteristics of Sputum Samples in the Three Groups

	Healthy children (n = 31)	Atopic asthma (n = 60)	Non-atopic asthma (n = 17)	*P* value
Sample[1]	24 (77.4%)	52 (86.7%)	12 (70.6%)	0.25
No contamination[1]	21 (87.5%)	51 (98.1%)	11 (91.7%)	0.16
Samples with viability ≥60%[1]	19 (79.2%)	45 (86.5%)	7 (58.3%)	0.08
Viability of cells median (IQR)	76% (70–84%)	81% (72–86,5%)	83% (69–87%)	0.27
Adequate sputum samples[1]	18 (75.0%)	42 (80.8%)	7 (58.3%)	0.27

[1]Results are expressed as absolute and percent numbers. Viability of cells is expressed as the median and IQR (interquartile range).

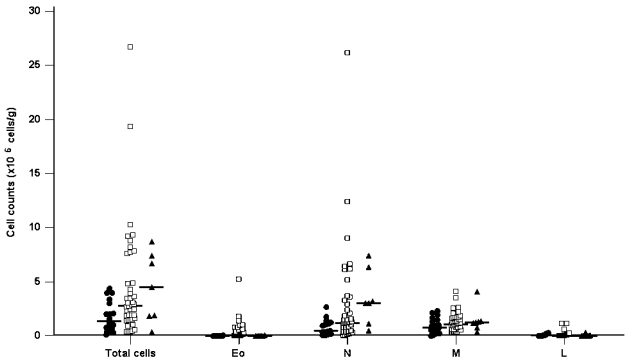

Fig. 1. Induced sputum cell counts in healthy controls (closed circles), atopic asthmatic children (open squares), and non-atopic asthmatic children (closed triangles). Eo, eosinophils; N, neutrophils; M, macrophages; L, lymphocytes. Horizontal bars represent the median values. Total cell count: $P < 0.05$ atopic asthma versus controls. Eosinophils: $P < 0.05$ atopic asthma versus controls and non-atopic asthma. Neutrophils: $P < 0.05$ non-atopic asthma versus controls.

There were no differences in the sputum neutrophil percentage between the three groups, but NAA children showed a higher median absolute neutrophil number (3.03×10^6 cells/g [0.65–5.59]) than the controls (0.42×10^6 cells/g [0.1–1.06]; $P = 0.003$). Lymphocyte percentage was higher in the controls than in either patient group, with no differences between atopic and NAA (Fig. 2), but there were no differences in the absolute lymphocyte number between the three groups. No differences were found in the cell profile or characteristics of induced sputum between asthma patients treated with ICS and untreated patients.

Cytokines in Induced Sputum

Induced sputum cytokine concentrations in the patients studied are shown in Figure 3. IFN-γ, IL-2, and IL-8 concentrations were higher in the two asthma groups than in the controls ($P < 0.05$), and IFN-γ and IL-2 were more highly elevated in AA than in NAA ($P < 0.05$). Concentrations of IL-4, IL-5, and IL-12p70 were higher in the AA group than in NAA patients or controls ($P < 0.05$). IL-10 concentration was lower in children with asthma (AA and NAA) than in the controls ($P < 0.05$), with no differences between the asthma groups.

Effect of Anti-Inflammatory Treatment

Twelve of the AA children with valid sputum samples and one NAA child were receiving inhaled corticosteroids. There were no significant differences in induced sputum cell count, cell profile, or cytokine values between AA children receiving or not inhaled corticosteroids. A separate analysis excluding children receiving this treatment and comparing cell count, cell profile and cytokine levels in the three groups did not vary the results; the same statistical differences described above were found.

DISCUSSION

In the present study, induced sputum Th1 and Th2 cytokine levels are compared between children with stable atopic or NAA and HC. We found that in the AA group, induced sputum levels of Th2 cytokines (IL-4 and IL-5) and sputum eosinophils were higher than in non-atopic patients and HC. We found no correlation

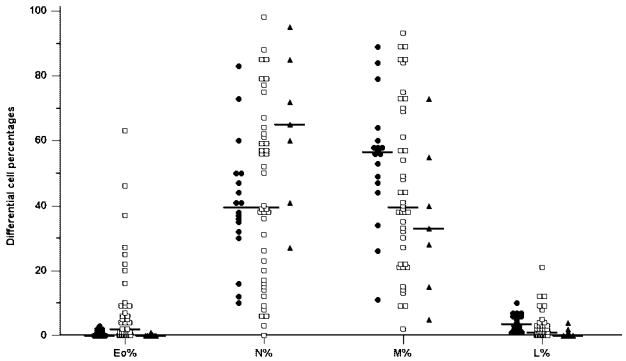

Fig. 2. Induced sputum cell profile in healthy controls (closed circles), atopic asthmatic children (open squares), and non-atopic asthmatic children (closed triangles). Eo%, percentage eosinophils; N%, percentage neutrophils; M% percentage macrophages; L%, percentage lymphocytes. Horizontal bars represent the median values. Eosinophils: $P < 0.05$ atopic asthma versus controls and non-atopic asthma. Lymphocytes: $P < 0.05$ atopic asthma versus control and non-atopic asthma versus controls.

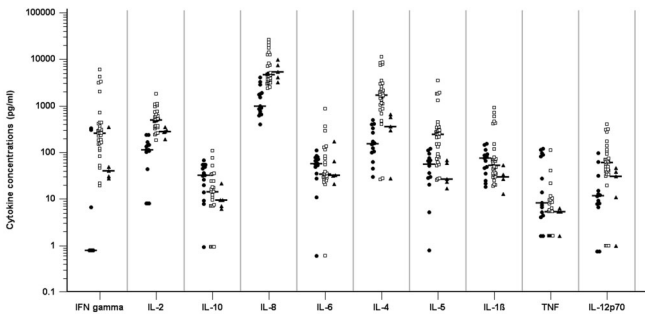

Fig. 3. Cytokine concentrations in induced sputum (pg/ml) in healthy controls (closed circles), atopic asthmatic children (open squares), and non-atopic asthmatic children (closed triangles). Horizontal bars represent the median values. IFN-γ, IL-2, and IL-8: $P < 0.05$ atopic and non-atopic asthma versus controls. IFN-γ and IL-2: $P < 0.05$ atopic versus non-atopic asthma. IL-4, IL-5, and IL-12p70: $P < 0.05$ atopic asthma versus controls and non-atopic asthma. IL-10: $P < 0.05$ controls versus atopic and non-atopic asthma.

between IL-5 concentration and eosinophil counts in sputum. Th1 cytokine concentrations (IFN-γ and IL-2) were higher in the two asthma groups than in the controls, and were highest in AA patients, an unexpected result. The chemokine IL-8 was elevated in asthma patients relative to HC, with no differences between atopic and NAA. IL-12p70 levels were also higher in atopic asthmatic patients than in the controls and non-atopic patients. None of the cytokines reached a higher concentration in NAA children than in atopic patients.

These findings support the notion that the Th1–Th2 balance is highly complex in asthma and that the inflammatory phenotype in patients is much more complex than a prevalence of Th1 or Th2 response depending on atopy status. Recent studies have offered a more multifaceted vision of the regulatory mechanisms governing the inflammatory response, in which the Th1 and Th2 pathways are interrelated and share cytokines, and where other recently described T helper subtypes, Th17, Treg, and Th9, are also implicated.[22] Furthermore, recent work has indicated that asthma phenotypes in children may not be stable; that is, inflammatory patterns in the same child may show alternating changes.[23] In this sense, Fleming et al.[23] found no differences in terms of atopy or asthma severity between children with eosinophilic and non-eosinophilic phenotypes. These considerations together with our results for cytokine levels lead us to think that atopic/NAA and eosinophilic/non-eosinophilic asthma may only be approximations of asthma phenotypes in children. The patterns of cytokine production along with cellularity in sputum of asthmatic children should define more precisely the different asthma phenotypes.

The higher levels of IFN-γ and IL-2 in AA patients were an unexpected result. Nonetheless, some studies in adults have shown that IFN-γ, a cytokine considered representative of the Th1 pathway, is elevated in atopic and NAA.[24] These results, together with the absence of IFN-γ that we found in HC, has prompted us to formulate the hypothesis that IFN-γ notably contributes to the pathogenesis of atopic and NAA in children.

Another important result was that differential cell count showed a higher percentage and higher total number of eosinophils in AA than in NAA or control children. None of the non-atopic patients and 45.5% of atopic patients presented induced sputum eosinophilia. We expected that more children with AA would have sputum eosinophilia and believe our findings can be attributed to two factors: a high presence of mild asthma in the atopic group (60%) and the effect of ICS treatment in some patients. One of the control children presented eosinophilia (sputum eosinophils 3%). The child was atopic and the eosinophilia could be a marker of future asthma. In keeping with the findings of Drews et al.,[9] inflammation was predominantly neutrophilic (in absolute number) in our children with NAA. The neutrophil percentages in asthmatic children and controls in our study triple the values reported by Drews et al.,[9] but are quite similar to those reported by Gibson et al.,[1] Cai et al.,[4] and Kulkarni et al.[27] The differences in neutrophil percentage in the related studies may be explained by differing backgrounds of the populations and by environmental factors. One unexpected finding from the present study was the higher percentage of lymphocytes in healthy controls than in asthma patients. The total lymphocyte count was, however, virtually identical in AA and control children.

The total cell count was higher in AA than controls, and showed a (non-significant) trend to higher values in NAA. These findings indicate that cell recruitment occurs in stable asthma and determines the inflammation present.

In the comparison of patients according to asthma severity, no differences were found in induced sputum cellularity or cytokine concentrations.

Technical issues related to cytokine measurement in sputum can complicate the interpretation of the results. There is some controversy regarding the use of DTT. Some authors have reported that DTT can change the concentration of certain cytokines,[21] whereas others have found no effect of DDT on some cytokine concentrations.[25] Furthermore, addition of a protease inhibitor might increase the measured level of cytokines. Simpson et al.[26] found that including a protease inhibitor in the sample processing raised IL-5 levels by 24%. In our study, samples were not treated with a protease inhibitor, and DDT was used. Nonetheless, all samples were treated in the same way. Thus, if there had been an effect, samples from all the patient groups would have been equally affected. In addition, to avoid an influence of the amount of sputum obtained and processed from each patient on the final cytokine levels, the volume of all samples was proportional to their weight so that the concentration of cytokines was not affected by possible dilution.

One potential limitation of our study is the fact that that some patients (38% in the AA group and 24% in NAA) were on ICS treatment with a wide dosing range (200–1,500 μg budesonide equivalent). ICS treatment can modify the asthma phenotype, including sputum cellularity and cytokine profile. We did not find differences in the inflammation markers between patients with and without corticosteroid treatment, although the numbers of patients available for analysis were small. Another limitation of our study is the small number of NAA patients included, which may have been an obstacle to revealing differences in certain cell counts and cytokine concentrations between the groups. A larger sample of children with NAA and controls would have

helped to better define differences between the patient populations.

In conclusion, the asthma phenotypes in atopic and non-atopic children, based on the induced sputum profile of Th1 and Th2 cytokines, showed an increase in both Th1 and Th2 cytokines in AA and a no clearly defined profile in NAA. These findings further support the concept that the inflammation present in atopic and NAA is much more complex than a simple balance between Th1 and Th2 responses. Future studies focussed on the cytokines implicated in asthma will be an aid to better understanding of the pathophysiology of this disease at the cellular and molecular levels.

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