

ORIGINAL ARTICLE

Bronchial hyperresponsiveness to methacholine and adenosine 5'-monophosphate, and the presence and degree of atopy in young children with asthma

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Clinical & Experimental Allergy

Summary

Background Bronchial hyperresponsiveness (BHR) is a characteristic feature of asthma, and is usually measured by bronchial challenges using direct or indirect stimuli. The relationship between atopy and BHR remains to be clarified, particularly in a population selected for asthma. Furthermore, data for young children are limited, although asthma frequently occurs in early childhood.

Objective The aim of this study was to investigate methacholine (direct stimulus) and adenosine 5'-monophosphate (AMP) (indirect stimulus) responsiveness according to the presence and degree of atopy in young children with asthma.

Methods A retrospective analysis of data from 122 preschool children (median age [range]: 5.3 years [4.0–6.8]) presenting with the diagnosis of asthma was performed. These children were characterized by skin-prick tests (SPTs) and bronchial challenges with methacholine and AMP, using a modified auscultation method. The end-point concentration, resulting in audible wheezing and/or oxygen desaturation, was determined for each challenge. Atopy was defined by at least one positive reaction to SPTs, and its degree was assessed using serum total IgE levels, number of positive SPTs, and atopic scores (sum of graded weal size).

Results Atopic patients ($n = 97$) had a significantly lower AMP end-point concentration than non-atopic patients ($n = 25$), whereas the methacholine end-point concentration was not different between the two groups. Among the atopic patients, there was no association between the methacholine end-point concentration and any of the atopy parameters. By contrast, a significant association was found between the AMP end-point concentration and the degree of atopy reflected in serum total IgE and atopic scores (χ^2 test for trend, $P = 0.001$, 0.003 , respectively).

Conclusion and Clinical Relevance Young children with atopic asthma had a significantly greater AMP responsiveness than those with non-atopic asthma, whereas methacholine responsiveness was not significantly different between the two groups. The degree of atopy appeared to be an important factor in AMP responsiveness, but not in methacholine responsiveness, and thus might be a marker of airway inflammation in asthma.

Keywords adenosine 5'-monophosphate (AMP), asthma, atopy, methacholine, young children
Submitted 17 March 2010; revised 18 October 2010; accepted 25 October 2010.

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Introduction

Bronchial hyperresponsiveness (BHR), defined as an exaggerated bronchoconstrictive response of the airways to a variety of stimuli, is considered to be the hallmark of asthma [1]. BHR is most commonly evaluated using methacholine or histamine, which acts directly at the level

of bronchial smooth muscle. However, BHR can also be assessed using indirect stimuli, such as adenosine 5'-monophosphate (AMP), which causes bronchoconstriction by stimulating or enhancing the release of mediators from mast cells [2]. There is an increasing interest in the role of AMP as a bronchoconstrictor stimulus, because it has been suggested that BHR to AMP is a better marker of

airway inflammation, particularly allergic inflammation, compared with direct stimuli such as methacholine [3].

Many studies have shown an association between atopy and BHR. Methacholine challenge is suggested to be more useful to identify clinically diagnosed asthma in atopic rather than non-atopic subjects [4]. In epidemiologic studies, atopy has constituted a risk for BHR to methacholine in children and adults [5, 6]. However, this relationship remains controversial in a population selected for asthma [7–9]. Further, it is not clear whether BHR to AMP is associated with atopy in patients with asthma. Some studies have suggested that atopy is critically important to the bronchial response to AMP [7, 10], whereas others found no difference in bronchial reactivity to AMP, based on the presence of atopy [9].

Most studies assessing the association between atopy and BHR considered atopy as a simple dichotomous variable, assigning individuals as either 'atopic' or 'non-atopic'. However, the quantification of atopic sensitization among atopic subjects may yield additional information on the relationship between atopy and BHR, as the demonstration of this relationship by using quantitative measures of atopy would clarify the concept that atopic sensitization has an impact on BHR.

The first attack of asthma frequently occurs within the first few years of life [11]; 80% of children develop symptoms before the age of 5 years [12]. A few studies have examined the relationship between the degree of atopy and BHR profiles for methacholine and AMP in patients with asthma [9, 13, 14]. However, data in young children are limited, although asthma frequently occurs in early childhood. The purpose of the present study was to investigate methacholine and AMP responsiveness according to the presence and degree of atopy in preschoolchildren with asthma.

Methods

This study was a retrospective analysis of the database of asthmatic children who had been challenged with methacholine and AMP for inclusion in clinical studies at our centre [15–17].

All subjects had the typical clinical picture of recurrent episodes of asthma symptoms (cough, wheezing, and dyspnoea) responsive to β_2 -agonists and symptom-free intervals. The symptom pattern was variable, including children with atopic or viral episodic symptoms; no attempt was made to delineate these phenotypes precisely. They needed intermittent use of β_2 -agonists with or without continuous use of low-dose inhaled corticosteroids, 500 μ g budesonide per day via nebulizer, to control their symptoms. To be eligible for the study, the patients were required to have more than four episodes of wheezing during the previous year, with at least one confirmed by a physician, and to meet the criteria for a positive asthma

predictive index [18]. These included either one major risk factor (a parental history of asthma or a personal history of atopic dermatitis) or two of three minor risk factors (allergic rhinitis, eosinophilia, and wheezing without colds). Clinical examination and chest radiographs were performed to exclude other rare causes of wheezing or clinically significant medical disorders apart from wheezing or allergy. Patients with a history of near-fatal asthma or major exacerbations necessitating the use of systemic corticosteroids were excluded.

Skin-prick testing (SPT) was performed using 12 common aeroallergens: house dust mites (HDM) (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), animal danders (cat epithelia, dog epithelia), pollens (mugwort, rye grass, ragweed, alder, oak), moulds (*Aspergillus fumigatus*, *Alternaria alternata*), and cockroach (*Blattella germanica*). Histamine and isotonic saline were used as positive and negative controls, respectively. Positive SPTs were defined by a mean weal diameter ≥ 3 mm larger than the negative control at 15 min after administration and atopy by the presence of at least one positive SPT. In addition to the number of positive SPTs, the weal size of positive SPT was also assessed. Weal diameters were graded so that a weal of 3.0–5.4 mm scored 1, 5.5–8.4 mm scored 2, 8.5–11.4 mm scored 3, and ≥ 11.5 mm scored 4; the scores were summed to give an atopic score for each subject.

Blood samples were collected from all subjects for measurement of total eosinophil counts and total IgE levels. The number of eosinophils was counted using an automated haematology analyzer (Coulter Counter STKS; Beckman Coulter, Fullerton, CA, USA) in a sample of blood containing ethylenediaminetetraacetic acid (EDTA). Serum total IgE levels were determined using the Coat-A-Count Total IgE IRMA (Diagnostic Products, Los Angeles, CA, USA).

Methacholine and AMP challenge tests were carried out using a modification of the method described by Springer et al. [19]. The two challenges were performed at the same time of day with an interval of 3–14 (median: 6) days. Their sequence was randomized to preclude any bias related to potential carry over effects. All subjects were asked to stop using antihistamines, inhaled bronchodilators, and other medications for 48 h and inhaled corticosteroids for 7 days before the tests. None of the subjects exhibited any symptoms of an upper respiratory infection during the month preceding the tests. Fresh solutions of methacholine (0.03–8 mg/mL) and AMP (0.39–200 mg/mL) were made up in a phosphate-buffered solution. All children were required to have normal chest auscultation and an arterial oxygen saturation of $\geq 98\%$ at baseline. Chest and tracheal auscultation was performed using a regular paediatric stethoscope by two paediatricians, and oxygen saturation was measured by pulse oximetry (Radical[®]; Masimoset Co., Irvine, CA, USA). The solutions

were delivered during quiet tidal breathing via a nebulizer (DeVilbiss 644; DeVilbiss, Somerset, PA, USA) driven by compressed air at a flow rate of 4.5 L/min; the unit was calibrated to give a mean output of 0.36 mL/min. Inhalation was performed using a face mask and continued for 2 min of tidal breathing, starting with the buffer solution. This was followed by doubling concentrations of methacholine or AMP every 5 min. After the end of each period of inhalation, the trachea and both lungs were auscultated repeatedly during quiet breathing for about 3 min. The two examiners assessed the wheezing independently, and wheezing was considered to occur only when their findings coincided. Arterial oxygen saturation was monitored continuously during and after each period of inhalation. The procedure was continued until either an end-point or the maximal concentration was reached. The end-point was defined as the concentration of methacholine or AMP resulting in audible wheezing and/or a fall in oxygen saturation of at least 5% from baseline (desaturation).

The institutional Review Board approved the studies [15–17] in which these subjects were initially enrolled, and written informed consent for participation in the original studies was obtained from parents of each subject.

Statistical analysis

Data are presented as the mean $\pm$ SD or as geometric means with a range of 1 SD. End-point concentrations were log-transformed before statistical analysis. Subjects were considered to have BHR to methacholine or to AMP when they had end-point concentrations of methacholine ≤ 8 mg/mL [19] or of AMP ≤ 200 mg/mL [20]. Censored values of 16 mg/mL for methacholine and 400 mg/mL for AMP were given to those that did not show a positive response after inhalation of the maximal concentration of methacholine (8 mg/mL) or AMP (200 mg/mL). The variables or frequencies were compared between the two groups, using the Student two-tailed *t*-test or the χ^2 test. Correlations between methacholine or AMP end-point concentration and blood eosinophil counts were calculated using the Pearson correlation test. The χ^2 test for trend was used to evaluate potential relationships between the degree of each atopy parameter (serum total IgE, number of positive SPTs, and atopic score) and methacholine or AMP end-point concentration. A *P*-value of 0.05 or less was considered to be significant.

Results

A total of 125 patients that had documented SPTs and methacholine and AMP challenges, were identified from the database. Three of these patients were excluded, because they did not meet the criteria of a positive asthma

predictive index. One hundred and twenty-two patients were therefore included in the final analysis.

On the SPTs, 97 patients (79.5%) had a positive response to at least one of the allergens, and thus were considered to have atopy. Among the individual allergens, HDMS were the most prevalent (*D. farinae* 74.6% and *D. pteronyssinus* 73.8%), followed by dog epithelia (27.9%), *B. germanica* (14.8%), cat epithelia (13.9%), *A. alternata* (12.3%), ragweed (11.5%), mugwort (9.8%), alder (9.8%), *A. fumigatus* (9.8%), oak (9.0%), and rye grass (6.6%). Of atopic subjects, 31 (32.0%) reacted only to HDMS, 4 (4.1%) only to allergens other than HDMS, and 62 (63.9%) to both categories. The mean wheal size was greatest for HDMS (*D. pteronyssinus* 8.1 ± 3.1 mm and *D. farinae* 6.9 ± 2.3 mm) (other allergens' data not presented).

One hundred and nineteen patients (97.5%) had BHR to methacholine, whereas 108 patients (88.5%) had BHR to AMP. BHR to both methacholine and AMP was detected in 106 patients (86.9%). Thirteen patients with BHR to methacholine failed to respond to AMP, whereas two patients with BHR to AMP failed to respond to methacholine. One patient responded to neither methacholine nor AMP.

In the patients with BHR to methacholine, the end-point concentration was determined by wheeze alone in 62 subjects, by both wheeze and oxygen desaturation in 27 subjects, and by oxygen desaturation alone in 30 subjects. In the patients with BHR to AMP, the end-point concentration was determined by wheeze alone in 56 subjects, by both wheeze and oxygen desaturation in 24 subjects, and by oxygen desaturation alone in 28 subjects.

The subjects were divided into two groups, according to the presence of atopy (Table 1). The atopic ($n = 97$) and non-atopic ($n = 25$) groups did not differ in terms of age, sex ratio, the use of inhaled corticosteroids, and blood eosinophil counts. The atopic group had a significantly lower AMP end-point concentration than the non-atopic group ($P = 0.000$), whereas methacholine end-point concentration was not significantly different between the two groups ($P = 0.447$). Serum total IgE levels were significantly higher in the atopic group than in the non-atopic group ($P = 0.000$).

To evaluate the association between the degree of atopy and bronchial responsiveness, subjects were stratified according to atopy parameters [serum total IgE levels (< 200 , 200–500, > 500 IU/mL), number of positive SPTs (1–2, 3, 4, 5 $\leq$), and atopic score (1–4, 5–6, 7 $\leq$)] and methacholine end-point concentration (Fig. 1) or AMP end-point concentration (Fig. 2) was assessed. The χ^2 test for trend showed that the methacholine end-point concentration was not associated with any of the atopy parameters (serum total IgE: $P = 0.146$; number of positive SPTs, $P = 0.468$; atopic score, $P = 0.217$) (Fig. 1). In the analysis of the AMP end-point concentration according to the different levels of the atopy parameters, a significant

association was found between serum total IgE or atopic scores and the AMP end-point concentration (χ^2 test for trend, $P=0.001$ and 0.028 , respectively), but not between the number of positive SPTs and the AMP end-point concentration ($P=0.212$) (Fig. 2).

There was an inverse correlation between the AMP end-point concentration and blood eosinophil counts ($r=-0.250$, $P=0.006$), but not between the methacholine

end-point concentration and blood eosinophil counts ($r=-0.097$, $P=0.290$) (data not shown).

Discussion

In the present study, preschoolchildren with atopic asthma had a significantly lower AMP end-point concentration than those with non-atopic asthma, whereas the methacholine end-point concentration was not significantly different between the two groups. Furthermore, among the atopic patients, the AMP end-point concentration, but not the methacholine end-point concentration, was found to be significantly associated with the degree of atopy, reflected in serum total IgE levels and atopic scores.

Asthma onset frequently occurs in early childhood [11, 12]. However, it is often difficult to make a definitive diagnosis until the child is older, because there is no lung function method that would be regarded as the gold standard for the diagnosis of asthma. In the present study of preschoolchildren, we chose a history of recent recurrent wheezing relieved by β_2 -agonist as one criterion for the diagnosis of asthma. This history has been proposed to constitute the basis for a diagnosis in settings where other causes have been excluded and the presence of asthma is likely [21]. The other criterion used was a positive asthma predictive index. It has been demonstrated that this index can predict the subsequent development of asthma with reasonable accuracy among children that have wheezing episodes during the first 3 years of life [18]. The diagnosis of asthma in our study population, therefore, is considered to be reasonably accurate.

Table 1. Clinical and atopy parameters of the two groups divided according to the presence of atopy

	Atopy (<i>n</i> = 97)	Non-atopy (<i>n</i> = 25)
Age (years)*	5.4 (4.0–6.7)	5.2 (4.2–6.8)
Sex (M/F)	61/36	16/9
Use of inhaled corticosteroids, <i>n</i> (%)	46 (47.4)	10 (40.0)
Blood eosinophils (/ μ L) [†]	459 $\pm$ 316	402 $\pm$ 362
Methacholine end-point concentration [‡]	1.19 (0.36–3.95)	1.43 (0.49–4.22)
AMP end-point concentration [‡]	20.61 (3.91–108.57) [§]	89.50 (25.38–315.67)
Atopy parameters		
Serum total IgE (IU/mL)	292.6 (102.5–835.5) [§]	59.3 (17.7–198.6)
Number of positive SPTs [†]	3.44 $\pm$ 1.94	–
Atopic score [†]	5.80 $\pm$ 2.86	–

*Median (range).

[†]Mean $\pm$ SD.

[‡]Geometric mean (range of 1 SD).

[§] $P<0.001$ vs. non-atopy.

AMP, adenosine 5'-monophosphate; SPT, skin-prick test.

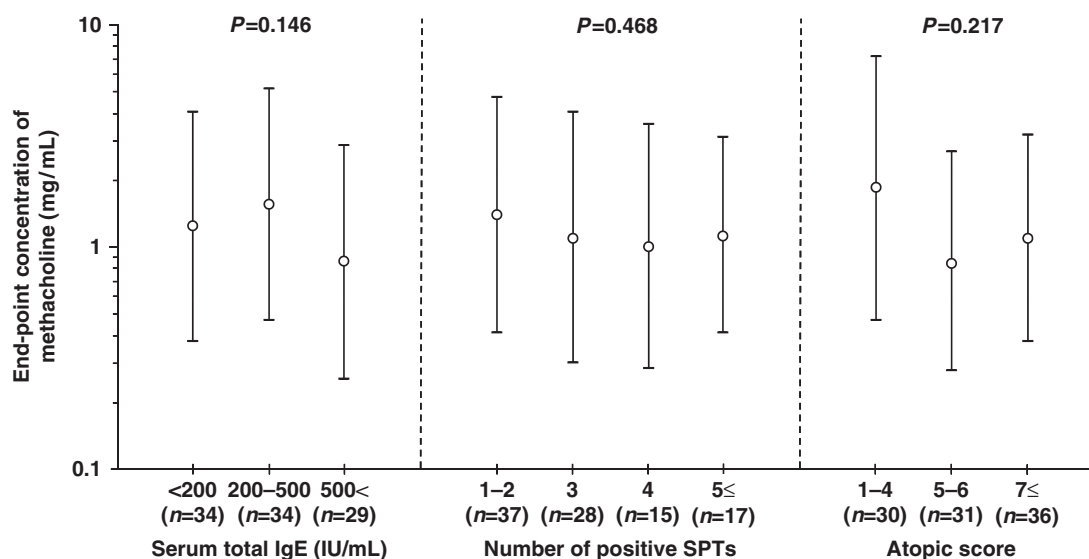


Fig. 1. End-point concentration of methacholine (geometric mean and range of 1 SD) according to the level of serum total IgE (left panel), number of positive skin-prick tests (SPTs) (middle panel), and atopic score (right panel). P values calculated by the χ^2 test for trend.

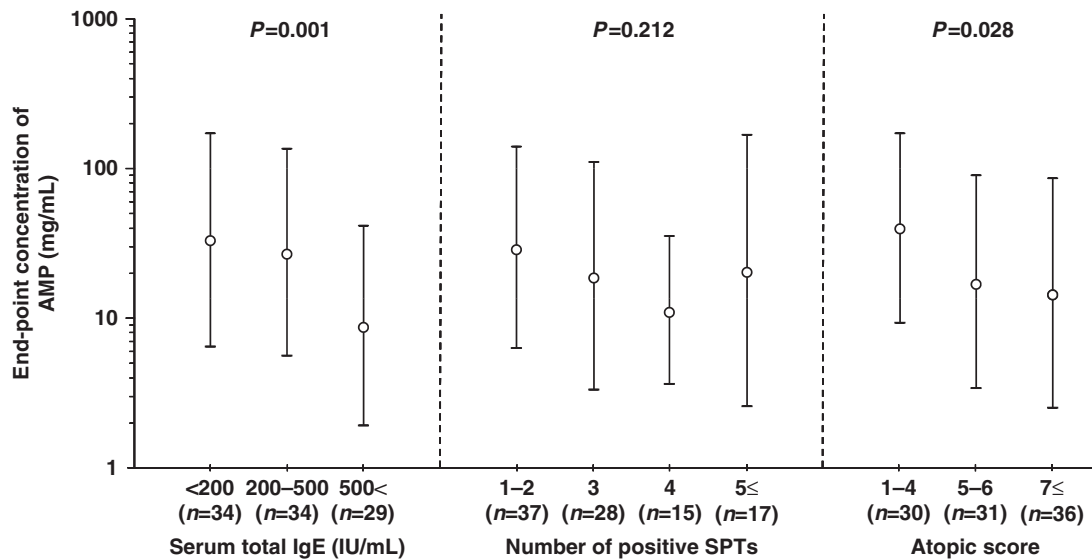


Fig. 2. End-point concentration of adenosine 5'-monophosphate (AMP) (geometric mean and range of 1SD) according to the level of serum total IgE (left panel), number of positive skin-prick tests (SPTs) (middle panel), and atopic score (right panel). *P* values calculated by the χ^2 test for trend.

Previously published rates of aeroallergen sensitization in young children with asthma have ranged from 33% to 86% [22, 23]. The relatively high percentage of atopic subjects (79.5%), in the present study, may be attributed to the fact that a positive asthma predictive index was included as a criterion for enrollment. HDMs were the most prevalent sensitizing agents and their weal size was the largest among the aeroallergens. These findings suggest that HDMs are the major local aeroallergen, consistent with the findings reported in the temperate regions like our country [24].

In the present study, bronchial challenges were performed using a modified auscultation method that set the two criteria (wheeze detection and oxygen desaturation) for determining the end-point. For both methacholine and AMP challenges, the incidence of wheeze detection at the end-point of a positive challenge was higher than that of oxygen desaturation. This is consistent with findings of other studies [19, 25] and suggests that wheeze detection may be a more sensitive indicator of induced bronchoconstriction than oxygen desaturation. In the present study, BHR to methacholine was found in 119 patients (97.5%), with a cut-off end-point concentration of 8 mg/mL [19, 25]. On the other hand, BHR to AMP was detected in 108 patients (88.5%) with a cut-off end-point concentration of 200 mg/mL [20]. This frequency was significantly lower than that of BHR to methacholine, suggesting that the latter is more sensitive than the former for diagnosing asthma in young children, which is consistent with the data in adults [26]. The nebulizer output (0.36 mL/min) in our provocation test protocol is more than twice as much as the guidelines (0.13 mL/min) recommended by the ATS [27]. Therefore, the cut-off

points (8 mg/mL for methacholine and 200 mg/mL for AMP), which were used to define BHR in the present study, may appear to be high, if these were for provocative concentrations causing a 20% fall in FEV₁ (PC₂₀s) in provocation tests using spirometry. However, this is not so, because PC₂₀ occurs at a lower concentration than end-points determined by wheeze detection and/or oxygen desaturation [28].

The two challenge tests were performed in a randomized fashion. It is unlikely that the first test would have influenced the second test, because the challenge days were separated by at least 3 days. It has been demonstrated that in sensitized patients with asthma, airway responsiveness to methacholine or AMP increases during the pollen season [29]. Even in patients sensitized to perennial allergens, such as HDMs, the seasonal variability of bronchial responsiveness to methacholine has been reported [30]. However, the time of the challenge tests could not be controlled in this study, because of its retrospective nature.

There are a few prior studies that compared methacholine and AMP responsiveness with regard to their relationship with atopy. Ludviksdottir et al. [7] observed that patients with atopic asthma are more hyperresponsive to AMP compared with patients with non-atopic asthma, whereas the frequency of BHR to methacholine was not different in the two groups studied. In young adults that were mostly asymptomatic, de Meer et al. [10] reported that BHR to AMP was associated with atopic airway diseases and atopic status, whereas BHR to methacholine was related to a reduced airway calibre. On the other hand, Fowler et al. [9] showed that the degree of skin-prick reactivity to aeroallergens was associated with BHR to methacholine, but not with BHR to AMP.

In the present study, the methacholine end-point concentration was not significantly different between the atopic and non-atopic groups. In addition, there was no significant association between the methacholine end-point concentrations and the degree of atopy, as measured by serum total IgE levels, number of positive SPTs, and atopic scores. These findings are consistent with other studies that reported no significant relationship between methacholine PC₂₀ and serum total IgE levels or the degree of skin-prick reactivity in children and young adults with asthma [13, 14]. The discrepancy between our results and those reported by Fowler et al. [9] are not easily explained, but possibly may be due to distinct environmental factors acting at different localities and/or different age groups studied. The results of this study suggest that mechanisms other than atopy are important in the pathogenesis of BHR to methacholine in young children with asthma.

There is increasing evidence suggesting that atopy is critically important in the bronchial response to AMP, apart from the studies by Ludviksodottir et al. [7] and de Meer et al. [10]. Currie et al. [31] showed that the distribution of allergic sensitization to HDMs, *Aspergillus*, cat allergen, and positive SPT numbers is significantly greater in asthmatic patients that are responsive to AMP than in non-responsive patients. Bakirtus and Turktaş [32] also reported that atopic sensitization is the most important predictive factor for BHR to AMP in school-age children with asthma, similar to adults [7, 10]. The same investigators subsequently studied preschool-age children with asthma [22], but failed to identify atopy as a predictor of BHR to AMP in this age group. These results, however, are difficult to interpret, because of their small sample size. In addition, the rate of atopy in their population was only 33.3%, which is even lower than the rates [33, 34] reported for patients with recurrent wheezing at the same age. Fowler et al. [9] found no association between either the presence or number of skin-prick positivities to aeroallergens and the degree of BHR to AMP. However, their results were inconclusive, because a high percentage of patients were using inhaled corticosteroid treatment, to which AMP responsiveness is very sensitive [35]. In the present study, the AMP end-point concentration was significantly lower in the atopic group than in the non-atopic group. Furthermore, a significant association was found between serum total IgE levels or atopic scores and the AMP end-point concentrations, even within atopic subjects. Because the vast majority of the subjects in our study had four or fewer SPT responses, this study probably lacked the contrast to demonstrate an effect of the number of positive SPTs on AMP responsiveness.

Most data on the relationships of methacholine or AMP responsiveness to atopy are based on the results obtained

from studies where bronchial challenges were performed using spirometry. This is the first study to compare methacholine and AMP responsiveness with regard to their relationship to atopy, using a modified auscultation method for bronchial challenges, in young children with asthma. The results of this study showed that AMP responsiveness was associated with the presence and degree of atopy as reflected in serum total IgE levels and atopic scores, whereas such a relationship was not observed for methacholine responsiveness. Our results may be explained in the light of an important role of mast cells in both atopy [36] and the airway response to AMP [2]. Mast cells are not of direct importance in the airway response to methacholine. Because the naturally occurring stimuli causing symptoms in asthma act through indirect mechanisms, it is hypothesized that indirect airway responsiveness should correlate better with clinical features of asthma, including severity and control, and current asthma activity [37]. This might provide a possible explanation for the difference in AMP responsiveness between atopic and non-atopic subjects. However, the proportion of subjects taking controller medications, i.e., inhaled corticosteroids, was comparable in the two groups, and other detailed clinical data were not available, due to the retrospective nature of this study. We have shown previously that while a majority of both atopic and non-atopic preschool wheezers were hyperresponsive to methacholine, atopic subjects were more hyperresponsive to AMP than non-atopic subjects [38]. The present study adds to the growing list of studies suggesting that atopy is critically important to the bronchial response to AMP, compared with methacholine.

Clinical studies in asthmatics have shown that BHR to AMP reflects underlying bronchial inflammation more accurately than BHR to methacholine [3]. This assertion is supported by our observation of a significant correlation between AMP end-point concentration and blood eosinophil counts, but not between methacholine end-point concentration and blood eosinophil counts. Other investigators have reported a similar finding [22]. We hypothesize that the degree of atopy may be a marker of airway inflammation in patients with asthma. This hypothesis is supported by a previous study that reported a correlation between serum total IgE levels and exhaled nitric oxide concentrations in allergic airway diseases including asthma [39].

In summary, young children with atopic asthma had a significantly greater AMP responsiveness than those with non-atopic asthma, whereas methacholine responsiveness was not significantly different between the two groups. Furthermore, the degree of atopy appeared to be an important factor in AMP responsiveness, but not in methacholine responsiveness, in these children.

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