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Airway inflammation in symptomatic and asymptomatic children with methacholine hyperresponsiveness

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ABSTRACT: A definition of asthma includes symptoms due to reversible airflow limitation and airway hyperresponsiveness. Characteristically, there is also airway inflammation. In children with methacholine airway hyperresponsiveness but no asthma symptoms, we examined whether there were features of asthmatic airway inflammation.

Forty one children, aged 11–16 yrs, were studied. Thirteen asymptomatic children with methacholine airway hyperresponsiveness (provocative concentration producing a 20% fall in forced expiratory volume in one second (PC_{20}) geometric mean of 3.35 (range 1.08–7.81) mg·ml^{-1}) were compared with 13 currently symptomatic asthmatics with a similar PC_{20} of 1.91 (0.42–6.5) mg·ml^{-1} and 13 normal children with a normal PC_{20} of 52.4 (17.6 to >64) mg·ml^{-1}.

Breathlessness experienced during a methacholine test was recognized to have occurred previously in 7 out of 13 asymptomatic children and all symptomatic children. Asymptomatic children had significantly more airway responses to hyperventilation with cold dry air (4 out of 13) than normal children (0 out of 13) but less than symptomatic children (11 out of 15). Sputum induced with hypertonic saline contained lower eosinophil counts in the asymptomatic children (median (interquartile range) 0.20 (0.59)%) than in the symptomatic children (1.70 (9.45)%), and not different from the normal children (0.15 (0.61)%). Budesonide, 400 μg *b.i.d.* improved respiratory symptoms, forced expiratory volume in one second (FEV_1) and methacholine PC_{20} in symptomatic children, but this effect did not reach statistical significance in asymptomatic children.

We conclude that symptomatic children are more likely to have evidence of asthmatic inflammation than asymptomatic children and this probably explains the symptom difference. However, a few asymptomatic children reacted to cold dry air, and some recognized that during the methacholine test they experienced similar breathlessness as they had in the past. These findings suggest a mild expression of symptoms in these asymptomatic children, probably due to mechanisms similar to those involved in asthma, but not identified as asthma.

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Methacholine airway hyperresponsiveness is associated with the presence of asthmatic symptoms in children [1–4], the presence and degree of variable airflow limitation [5], and the severity of asthma as assessed by diurnal variation of peak expiratory flow (PEF) [6], or by requirements for treatment [7]. Nevertheless, a proportion [6.7–33%] of children, show mild to moderate methacholine airway hyperresponsiveness without any history of previous or current asthma symptoms [1–4]. Some of these patients with asymptomatic methacholine airway hyperresponsiveness also have increased diurnal variability of PEF [8], like current symptomatic asthmatics.

Airway inflammation is a major factor in the pathogenesis of asthma [9]. It is characterized by mucosal oedema, desquamation of the epithelium, fibrosis beneath the basement membrane, and infiltration by eosinophils and mast cells [10]. Increased numbers of eosinophils and mast cells have been shown in bronchial biopsies [11–14], bronchoalveolar lavage [15, 16] and sputum [17, 18] of current asthmatics, compared to normal subjects.

Airway hyperresponsiveness to cold dry air is present in most asthmatics [19]. The mechanism is not fully understood, but is probably due to the release of mediators, and the test may be more specific for asthma than methacholine airway hyperresponsiveness. Therefore, hyperresponsiveness to cold dry air is likely to be associated with inflammatory cells in the airways, and might be expected in subjects with asymptomatic airway

hyperresponsiveness if they have airway inflammation.

Inhaled corticosteroid is a potent anti-inflammatory medication, and has been shown to improve respiratory symptoms and methacholine airway hyperresponsiveness in asthmatics [20, 21]. Bronchial biopsy studies have shown that inhaled corticosteroid reduces eosinophil and mast cell infiltration in the bronchial epithelium and mucosa [22, 23], suggesting that improvement in clinical and physiological indices is due to this anti-inflammatory action. The possible effect of inhaled corticosteroid on methacholine airway responsiveness is likely to reflect the initial presence of airway inflammation.

In this study, we wanted to determine whether methacholine airway hyperresponsiveness in asymptomatic children is associated with airway inflammation. We therefore compared children with asymptomatic methacholine airway hyperresponsiveness with currently symptomatic asthmatic children (with a similar degree of methacholine airway hyperresponsiveness) and with normal children with respect to: 1) airway hyperresponsiveness to isocapnic hyperventilation of cold dry air; 2) eosinophil and metachromatic cell counts in sputum induced by hypertonic saline; and 3) the effect of 2 weeks treatment with inhaled corticosteroid. We also examined whether asymptomatic hyperresponsive children were truly asymptomatic, by recording the occurrence of breathlessness during the methacholine test and whether this was recognized to have occurred in the past.

Methods

Subjects

Forty one children, aged 11–16 yrs, were selected from 77 volunteers of families of staff, and patients attending the Chest and Allergy Clinics at the Health Sciences Centre in Hamilton. Thirty six subjects were not included in the study, because they did not meet the clinical and physiological characteristics of the three groups. All were nonsmokers, and none had a respiratory tract infection or had been exposed to a seasonal allergen within one month. All had normal baseline forced expiratory volume in one second (FEV_1) greater than 70% predicted, and an FEV_1/vital capacity(VC) >75%, and all were able to perform FEV_1 reproducibly with a coefficient of variation <5%.

Presence of asthma symptoms was detected by a questionnaire, including questions previously used in epidemiological surveys and shown to be associated with clinical asthma [3, 24]. The relevant questions were: 1) Has your child had recurrent wheezy breathing with shortness of breath? 2) Has your child had recurrent attacks of shortness of breath that came on when he/she was at rest? 3) Has your child been recurrently woken up by wheezy breathing or shortness of breath? 4) Has your child ever had attacks of wheezing during or after exercise, when going out in cold air or during colds or respiratory infections? 5) Has your child ever been diagnosed as having asthma by a doctor or at the hospital?

Table 1. – Clinical physiological charactersistics of the subjects

	Normal children	Asymptomatic children	Asthmatic children
Subjects n	13	13	15
Gender M/F	5/8	4/9	11/4
Age* yrs	13.7(11–16)	13.5(12–16)	13.6(11–16)
Atopy+ μm	0(0–0.68)	1.71(0–3.16)	1.58(0–3.16)
Number of subjects with:			
family history of atopy	5	12	12
allergic rhinitis	3	11	14
recurrent cough	3	7	13
FEV_1 % pred§	116(10.8)	123(13.5)	112(12.7)
FEV_1/VC % pred§	102(7.8)	101(4.5)	103(7.9)
PC_{20} mg·ml^{-1}#	52.4(17.6–>64)	3.35(1.1–7.8)	1.91(0.4–6.5)

*: data presented as mean and range in parenthesis; +: data presented as median and range in parenthesis; §: data presented as mean and SD in parenthesis; #: data presented as geometric mean and range in parenthesis. Atopy: size of allergy prick tests is mean of mean wheal diameters of the response to 19 allergy skin tests. PC_{20}: provocation concentration of methacholine producing a 20% fall in FEV_1; FEV_1: forced expiratory volume in one second; VC: vital capacity.

Any child with one positive answer was considered to present with asthma symptoms.

Children were classified into three groups according to the presence or absence of asthma symptoms, and to the degree of airway responsiveness to methacholine. Thirteen were normal children (NC) who had never had symptoms of asthma and had normal methacholine airway responsiveness. Thirteen were asymptomatic children (AC), who had never had symptoms of asthma, but had methacholine airway hyperresponsiveness. Fifteen were symptomatic children (SC), with symptoms in the past 2 weeks, who had mild asthma treated only with β_2-agonists on demand and had methacholine hyperresponsiveness of a similar degree to the asymptomatic children. More asymptomatic children and symptomatic children than normal children had a family history of eczema, allergic rhinitis or asthma, and a personal history of allergic rhinitis (Fisher exact test p=0.003) or recurrent cough (Fisher exact test p=0.003). Atopy assessed by positive allergy skin prick tests (expressed as mean wheal diameter response to 19 allergy skin tests) was similar in the asymptomatic children and symptomatic children and higher than in the normal children (Kruskall-Wallis test p=0.0001) (table 1).

The study was approved by the McMaster Health Sciences Centre Research Ethics Committee and all of the children and their parents gave written informed consent.

Study design

Subjects attended the laboratory on 4 or 7 days. On visit 1, subject characteristics were documented by a

questionnaire, and allergy skin prick tests with 19 common allergens [25], and a methacholine inhalation test were performed. On visit 2, airway responsiveness to isocapnic hyperventilation of cold dry air was measured. On visits 3 and 4, sputum was induced. These four initial visits were performed within 3 weeks. Then the children in the AC and SC groups were asked to inhale budesonide (Pulmicort Turbuhaler®, 400 μg twice daily) and placebo of identical appearance, for 2 weeks in a randomized double-blind cross-over study, with a washout period of 2 weeks between treatments. At baseline and after each treatment period (visits 4–7), the severity of respiratory symptoms over the past 2 weeks and methacholine airway responsiveness were determined. The initial methacholine provocative concentration producing a 20% fall in FEV_1 (PC_{20}) was used as baseline for the first treatment period. The washout period could be extended up to 4 weeks if the methacholine PC_{20} was not back to within one doubling concentration of the baseline measurement. Compliance was assessed by counting the remaining doses in the turbuhaler at the end of each treatment period.

Methods

Severity of respiratory symptoms over the preceeding 2 weeks was assessed with nine questions from the respiratory symptom domain of the paediatric asthma quality of life questionnaire [26]. For the asymptomatic children the word "asthma" was replaced by "breathing". The response scale to each question was a seven point Likert scale from: 1= did not bother me; to 7= bothered me very, very much. The sum of the responses provided an asthma symptom score over the past 2 weeks; the minimum score was 9 and the maximum 63. To improve reproducibility and responsiveness of the questionnaire, children were reminded of their previous answers [27]. In addition, a global transitional scale was used to assess changes in overall severity of respiratory symptoms. The response was recorded as "worse", "the same" or "better".

Spirometry [28] and methacholine inhalation tests were performed as described by COCKCROFT *et al.* [29], and updated by JUNIPER *et al.* [30]. The result was expressed as the provocative concentration to cause a fall in FEV_1 of 20%. Methacholine airway responsiveness was considered in the normal range if the PC_{20} was >16 $mg \cdot ml^{-1}$ and in the hyperresponsive range if the PC_{20} was <8 $mg \cdot ml^{-1}$. At the end of the challenge, perception of breathlessness was assessed using a modification of the Borg scale [31, 32]. Subjects were instructed to select either whole numbers or fractions and to ignore other stimuli, such as nasal or throat irritation and cough. They were helped to recognize breathlessness by use of related words (tightness in the chest, discomfort or difficulty in breathing). At the same time they were asked if they had felt the same way before and, if yes, when and in which circumstances. Tests with isocapnic hyperventilation of cold dry air were performed as described by O'BYRNE *et al.* [19] and results were expressed as the provocative dose of respiratory heat exchange to cause a fall in FEV_1 of 10% (PD_{10}).

Induction of sputum by inhalation of hypertonic saline was performed as described by PIN *et al.* [18]. Briefly, after inhalation of terbutaline (Bricanyl® 1 mg), subjects inhaled increasing concentrations of hypertonic saline solution (3–5%) for 5 min periods up to 30 min, or less if an adequate sample was obtained. FEV_1 was recorded after terbutaline and every 5 min, to detect any bronchospasm induced by hypertonic saline solution. Every 5 min the subjects were asked to cough up secretions that might be present. Sputum analysis was carried out as described previously [17, 18]. Quality of the sample was rated from 0 to 6 according to the number of suitable lower respiratory tract plugs which were present, and to salivary contamination in cell counts. An adequate sample (allowing a differential cell count to be performed without salivary contamination) was defined by a score ≥4, an intermediate sample by a score of 3, and an inadequate sample by a score ≤2. The method was modified in one way; chromotrope 2R (C2R) replaced May Grünwald Giemsa (MGG) to stain eosinophils. In a formal study of 20 samples stained by both techniques and counted twice by two investigators, the counts performed with the C2R stain were shown to be easier, faster, similarly reproducible within investigators (coefficient of repeatability R=0.84 for C2R and 0.83 for MGG) and more reproducible between investigators (R=0.68 for C2R and 0.46 for MGG). Two slides were fixed with formalin and stained with C2R for differential cell counts of eosinophils, and 400 cells were counted per slide. Two other slides were fixed with Carnoy's solution and stained with 0.5% toluidine blue in 0.7 M HCl at pH 0.1 for differential cell counts of metachromatic cells and 1,500 cells were counted per slide. Slides were coded and counted blind to the clinical characteristics of the subjects by one investigator (RK). Cell counts obtained from two sputum samples of the same subject were averaged for analysis. When children had one inadequate sample, the results of cell counts from the other sample were used. When no adequate results were available, the values were missing.

Statistical analysis

Statistical analysis was performed using SAS (version 6.06). The PC_{20} values were log-transformed for the analysis and expressed as geometric mean and range. Cell count results were expressed as median and interquartile range, (IQR) other results as arithmetic mean and standard deviation. Comparison between groups were performed by χ^2 test and Fisher's exact test for 2 category data, and by Kruskall-Wallis test for more than 2 category and arithmetic data. If the p value was less than 0.1, analysis of variance (ANOVA) test on ranked scores was performed to contrast between two groups. Reproducibility of scores and cell counts was assessed by calculation of the coefficient of repeatability (R) derived from an ANOVA on repeated measures [33]. Effects of the treatment and carry-over effects were

analysed, in each group, by an analysis of variance model with patient, treatment, period and sequence as factors. Relationship between arithmetic data was analysed by Spearman rank correlation coefficient. P values <0.05 were considered statistically significant.

Results

Physiological characteristics

Spirometry, airway responsiveness to methacholine and isocapnic hyperventilation of cold dry air were compared in the three groups. Baseline spirometry (FEV_1, FEV_1/VC) was similar. Methacholine PC_{20} was not statistically different in the AC compared to SC group (geometric mean 3.35 (range 1.1–7.8) *vs* 1.91 (0.4–6.5) mg·ml^{-1}) (Student unpaired t-test p=0.06) and significantly lower than in NC group (52.4 (17.6 to >64) mg·ml^{-1}) (table 1). Subjective assessement of breathlessness at the end of the methacholine test was similar in the AC group compared to SC (Borg score mean 4.3 (SD 1.6) *vs* 5 (2)) for a similar fall in FEV_1 (28.9 (6.8) *vs* 33.1(14)%). Symptoms developing during the methacholine challenge were recognized as having been previously felt by all the symptomatic children, whereas seven of the asymptomatic children also reported to have previously experienced the symptoms. Respiratory heat exchange achieved at the end of isocapnic hyperventilation of cold dry air was similar in the three groups. Positive airway responses, as assessed by PD_{10}, were more frequent in the AC group (4 out of 13) than in the NC group (0 out of 13), but less than in the SC group (11 out of 15) (p=0.003) (fig. 1).

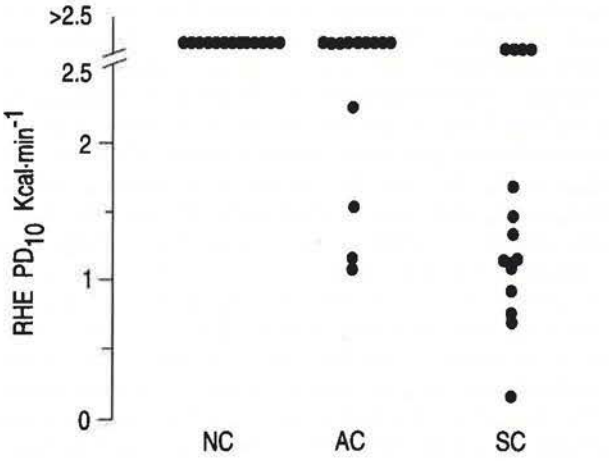

Fig. 1. – Respiratory heat exchange to cause a 10% fall in FEV_1 (RHE PD_{10}) during isocapnic hyperventilation of cold dry air in normal (NC), asymptomatic (AC) and symptomatic asthmatic (SC) children. FEV_1: forced expiratory volume in one second.

Induced sputum

Quality scores of the induced sputa were slightly higher in the SC groups with 89% adequate samples, 4% intermediate samples and 7% inadequate samples,

Table 2. – Sputum quality scores and eosinophil and metachromatic cell counts in induced sputum

Subject No.	Sputum quality scores			Eosinophils % of nucleated cells			Metachromatic cells % of nucleated cells		
	NC	AC	SC	NC	AC	SC	NC	AC	SC
1	3.0	5.0	6.0	0.30	0	0.40	0	0	0
2	3.0	4.5	3.0	0.90	0.97	12.53	0	0	0.67
3	6.0	5.5	5.0	0.05	0.20	13.40	0	0	0.15
4	3.0	5.0	6.0	0.65	0.10	9.85	0	0	0
5	4.0	5.5	4.5	0	0.15	12.00	0	0	0.05
6	4.0	5.0	3.5	0	0.30	8.00	0	0	0.05
7	3.5	4.5	2.0	0.60	0.65	*	0	0	0
8	4.5	5.5	5.0	0.15	0.70	0	0	0.20	0
9	6.0	2.0	5.5	0	0.20	2.00	0	0	0.02
10	6.0	1.5	4.5	0	0.15	1.40	0	0	0
11	4.5	4.0	5.0	0	0	1.15	0	0	0
12	6.0	3.5	5.0	2.15	0	1.35	0	0	0.27
13	6.0	5.5	5.0	0.70	2.15	0.25	0	0.15	0
14			4.0			0.20			0
15			5.0			6.55			0
Median				0.15	0.20	1.70	0	0	0
IQR				0.66	0.59	9.45		0	0.05
Statistics: *vs* NC					NS	p=0.004		p=0.3	p=0.009
vs AC						p=0.008			p=0.1

Quality scores: mean of 2 sputum inductions (cf text). NC: normal children; AC: asymptomatic children; SC: asthmatic children; IQR: Interquartile range; NS: nonsignificant; *: missing value. Statistical results are derived from Kruskall-Wallis tests with analysis of variance (ANOVA) test on ranked scores if the p value was less than 0.1.

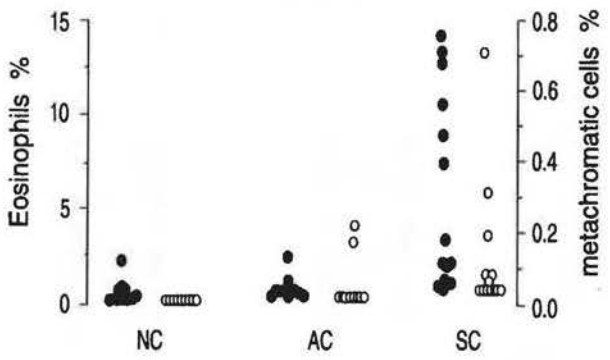

Fig. 2. – Differential counts of eosinophils (●) and metachromic cells (○) in induced sputum, expressed as percentage of nucleated cells, in normal (NC), asymptomatic (AC) and symptomatic (asthmatic) (SC) children. Each point represents the mean of 2 sputum samples counted for each subject.

compared with 73, 15 and 12% in the AC group and 68, 28 and 4% in the NC group (table 2). Reproducibility of cell counts was good for eosinophils (R=0.75) but less satisfactory for metachromatic cell counts (R=0.45). Eosinophil counts were lower in the AC group (median (IQR) 0.20 (0.59)%) than in the SC group (1.70 (9.45)%), and not different from the NC group (0.15 (0.61)%) (Kruskall-Wallis test p=0.009) (table 2, and fig. 2). Metachromatic cell counts showed a similar trend (two subjects with countable metachromatic cells in the AC group compared to six in the SC group and none in the NC group, p=0.03), but only the SC were statistically different from the NC.

There was a weak but statistically significant relationship between either eosinophil or metachromatic cell counts in induced-sputum and methacholine PC_{20} (correlation coefficient r=-0.51, p=0.008 and r=-0.36, p=0.025, respectively) for the whole group of subjects. There was no significant differences in either eosinophil or metachromatic cell counts between subjects with and without positive responses to isocapnic hyperventilation of cold dry air.

Among the AC group, we could not identify a subgroup of subjects who might present with some of the characteristics of asthma. Four subjects had neither positive airway responses to cold dry air nor previous perception of symptoms, or increased metachromatic cells, two had responses to cold dry air and previous perception of symptoms but no countable metachromatic cells, and seven had only one of these characteristics.

Effect of inhaled corticosteroid

Three asymptomatic children did not meet the washout period requirement, *i.e.* their methacholine PC_{20} did not return to baseline after 4 weeks. However, no discrepancies in statistical results were found from excluding these three subjects from the analysis; therefore, the results are given for the whole group. No carry-over effect was detected either on clinical scores, baseline measurements, or changes of FEV_1, FEV_1/VC, VC and PC_{20}. Mean compliance exceeded 90% for all periods.

Baseline symptom scores were higher in the SC group (mean (SD) 28 (8)) than in the AC group (16.8 (4.1)).

Table 3. – Effect of budesonide turbuhaler (400 mg twice daily for 2 weeks) *vs* placebo in a randomized cross-over controlled trial on FEV_1 (in % predicted), methacholine PC_{20}, asthma symptom scores and overall subjective assessement in the asymptomatic (AC) and in the symptomatic asthmatic (SC) children

	AC n=13						SC n=15					
	Placebo			Budesonide			Placebo			Budesonide		
	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ
FEV_1† % pred	123 (12.8)	123 (12.3)	-0.3 (3.2)	122 (12.8)	126 (12.3)	3.1 (5.5)	113 (12.9)	11 (16.4)	-2.7 (7.1)	112 (13.0)	114 (15.8)	1.9 (5.3)
			p=0.052						p=0.025			
PC_{20}* mg·ml^{-1}	4.8 (1.4–19.5)	7.2 (1.2–55.9)	2.5	3.9 (1.1–18.3)	12.1 (1.9–>64)	13.6	1.9 (0.5–10.3)	1.5 (0.4–10.2)	0.6	1.7 (0.3–7.8)	2.4 (0.4–22.1)	2.0
			p=0.09						p=0.018			
Asthma† symptom score	14.8 (4.3)	14.3 (5.7)	-0.5 (4.2)	16.2 (3.6)	15.2 (4.8)	-1 (3.9)	25.3 (8.8)	26.6 (8.9)	1.3 (5.3)	31.2 (10.1)	25.3 (9.4)	-5.9 (6.5)
			NS						p=0.009			
Overall subjective n W		0			0			4			1	
S		13			12			9			7	
B		0			1			2			7	

†: data presented as mean and SD in parenthesis: *: data presented as goemetric mean and range in parenthesis. Δ: differences between FEV_1 (or methacholine PC_{20} or symptom score) post-treatment minus FEV_1 (or methacholine PC_{20} or symptom score) pretreatment. P values represent the statistical significance of differences between effects of placebo and budesonide, calculated from ANOVA analysis. W: felt worse; S: felt the same; B: felt better. For further abbreviations see legends to tables 1 and 2.

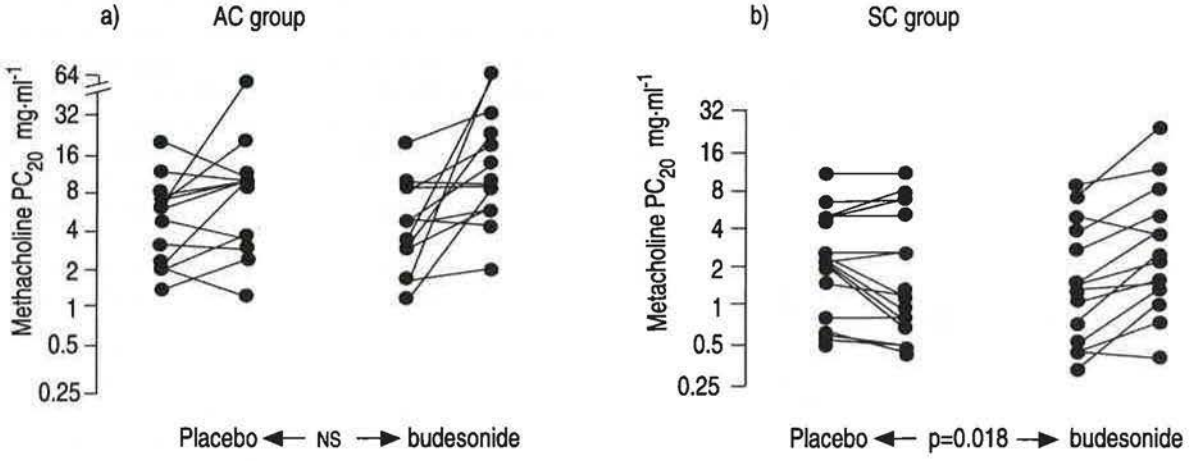

Fig. 3. – Effects of budesonide (400 μg twice daily for 2 weeks) *vs* placebo on methacholine PC_{20} in a) asymptomatic (AC) and in (b) symptomatic asthmatic (SC) children. PC_{20}: provocative concentration producing a 20% fall in forced expiratory volume in one second.

When improvement in clinical scores was analysed, there was a statistically significant difference between budesonide and placebo in the SC group (ANOVA p=0.009), but not in the AC group (p=0.74) (table 3). In the AC group only one subject felt better on budesonide; all others felt the same. In the SC group 7 felt better, 7 the same and 1 worse on budesonide, whereas 2 felt better, 9 the same and 4 worse on placebo. In the SC group there was a statistically significant difference between the effect of budesonide and placebo on methacholine PC_{20} (p=0.018), whereas the difference was not statistically significant in the AC group (table 3, Fig. 3). There was also a statistically significant effect of budesonide on FEV_1 in the SC group (p=0.025), whereas the effect in the AC group did not reach statistical significance (p=0.052) (table 3).

There was no significant relationship between changes in methacholine PC_{20} with budesonide in either group and either eosinophil or metachromatic cell counts in induced sputum (Spearman rank correlation coefficient r=0.13 and 0.01, respectively) or airway responses to isocapnic hyperventilation of cold dry air (Wilcoxon-signed rank test p>0.05) measured before the start of the treatment.

Discussion

Children with asymptomatic methacholine airway hyperresponsiveness, when compared with normal and currently symptomatic asthmatic children with a similar degree of methacholine airway hyperresponsiveness, showed an intermediate positive airway response rate to isocapnic hyperventilation of cold dry air. Asymptomatic children also differed from asthmatics, but resembled normals, in the numbers of eosinophils and metachromatic cells in induced-sputum, and the lack of significant improvement in methacholine airway hyperresponsiveness with inhaled corticosteroid compared to placebo.

This is the first study to examine airway inflammatory cell infiltration in children with asymptomatic methacholine airway hyperresponsiveness. The normal eosinophil and metachromatic cell counts in induced-sputum may suggest that there was no active inflammation. The absence of abnormal cells might reflect a different cause for the airway hyperresponsiveness, such as local release of mediators and cytokines by lymphocytes and fibroblasts [34], or structural changes in the airways, such as smooth muscle hypertrophy or subepithelial fibrosis, which can occur in atopic nonasthmatic subjects [35]. Alternatively, the normal sputum cell counts in some symptomatic children may indicate that induced-sputum cell counts may be an insensitive measurement, when abnormalities are milder or confined to the mucosa. Nevertheless, the counts were clearly lower in the asymptomatic than in the asthmatic children. Further investigation by bronchial biopsies and/or bronchial brushings will help to clarify this issue.

In our study, most of the subjects (26 out of 28) with methacholine airway hyperresponsiveness were atopic, as compared with only 4 out of 13 normal children. Previous studies have shown that atopic nonasthmatic subjects have higher prevalence of methacholine airway hyperresponsiveness than nonatopic subjects [36]. Therefore information coming from bronchial biopsies performed in atopic nonasthmatic subjects may help in understanding the relationship between asthmatic symptoms, inflammation and airway hyperresponsiveness. DJUKANOVIC *et al.* [37] showed some markers of airway inflammation (intermediate increased eosinophils and features of degranulation of eosinophils and mast cells similar to the asthmatics) in atopic nonasthmatics, whereas BRADLEY *et al.* [38] did not find either increase of cells or increased expression of activation markers in a similar group of subjects. However, in both studies, it was difficult to know how these findings related to methacholine airway hyperresponsiveness, as methacholine PC_{20} was within the asthmatic range in only a few subjects. Regardless of the basis for the methacholine airway hyperresponsiveness in the ACc children group, the absence of symptoms, together with the absence of inflammatory cells, suggests that inflammatory cells in sputum may be an important

determining factor for the clinical expression of asthma. The presence of airway hyperresponsiveness in the absence of clinical asthma may be secondary to previous episodes of inflammation which are no longer present.

Airway hyperresponsiveness to isocapnic hyperventilation of cold dry air was present in some asymptomatic children in our study. This finding was similar to results in atopic nonasthmatic adults, in whom DEAL *et al.* [39] and RAMSDALE *et al.* [40] found a proportion of subjects responding to hyperventilation of cold dry air, and suggested that they might have mild asthma. In stable asthmatics, airway hyperresponsiveness to cold dry air correlates with methacholine airway hyperresponsiveness [19], but is rare in smokers with chronic airflow limitation [41], and absent in children with nonasthmatic bronchial diseases [42], when methacholine airway responsiveness is increased. Thus, cold air airway hyperresponsiveness may be more specific for asthma than methacholine airway hyperresponsiveness. Inhaled corticosteroid improved methacholine PC_{20} when compared with placebo. There was a significant difference in the symptomatic children, but not in the asymptomatic children. This could be due to the fact that the methacholine PC_{20} varied much more with placebo in the asymptomatic group. We have no clear explanation for the latter finding, except that the group may represent a more heterogeneous population. However, the appropriateness of the difference in therapeutic effect was also indicated by improvement in symptoms and FEV_1 in symptomatic children. Reduction of methacholine airway hyperresponsiveness by inhaled corticosteroid is due, at least in part, to the reduction in inflammatory cells, including eosinophils and mast cells, in bronchial epithelium and mucosa [22, 23]. Asymptomatic subjects did not have increased numbers of inflammatory cells in their induced-sputum. These findings support the conclusion that infiltration with inflammatory cells is an important determinant of the clinical expression of asthma. Short-term inhaled corticosteroid does not affect collagen thickening beneath the basement membrane [23], and this type of finding could explain the lack of improvement in methacholine airway hyperresponsiveness, if this is a cause of hyperresponsiveness. Alternatively, airway hyperresponsiveness may be due to inflammation located elsewhere, as suggested by AUBIER *et al.* [43], who treated atopic subjects with allergic rhinitis and carbachol airway hyperresponsiveness but no symptoms of asthma with the same dose of topical corticosteroid given nasally or into the bronchi; airway hyperresponsiveness improved after nasal but not after bronchial administration of inhaled corticosteroid.

It is possible, that at least some of the asymptomatic children with methacholine airway hyperresponsiveness had mild asthma. Most of them presented with some characteristics of asthma, *i.e.* some recalled previous symptoms resembling those caused by methacholine, some had been troubled by recurrent cough, and others were hyperresponsive to cold dry air. GIBSON *et al.* [8] showed that children with asymptomatic methacholine airway hyperresponsiveness, selected in a similar way to those in the present study, had increased diurnal variability of peak expiratory flow (PEF) like that of asthmatics. These findings suggest a mild expression of symptoms in these asymptomatic children, probably due to mechanisms resembling those involved in asthma, but not identified as asthma.

In conclusion, the differences between asthmatic children and children with asymptomatic airway hyperresponsiveness were an increase in eosinophils and metachromatic cells in induced-sputum, and budesonide-induced improvement in symptoms and methacholine PC_{20} in symptomatic asthma. The reason for methacholine airway hyperresponsiveness without recognized symptoms of asthma remains unknown. Residual effects of previous inflammatory processes are possible. Clarification of the mechanisms of airway hyperresponsiveness in asymptomatic children will require prospective long-term studies, possibly with bronchial biopsies.

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