

Bronchoalveolar lavage findings suggest two different forms of childhood asthma

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Summary

Background It seems plausible that children with atopy and persistent asthma symptoms will, like their adult counterparts, have chronic airways inflammation. However, many young children with no other atopic features have episodic wheezing that is triggered solely by viral respiratory infections. Little is known as to whether airways inflammation occurs in these two asthma patterns during relatively asymptomatic periods.

Methods Using a non-bronchoscopic bronchoalveolar lavage (BAL) procedure on children presenting for an elective surgical procedure, this study has investigated the cellular constituents of BAL fluid in children with a history of atopic asthma (AA) non-asthmatic atopic children (NAA) or viral associated wheeze (VAW).

Results A total of 95 children was studied: 52 with atopic asthma (8.0 years, range 1.1–15.3, 36 male), 23 with non-asthmatic atopy (median age 8.3 years, range 1.7–13.6, 11 male) and 20 with VAW (3.1 years, range 1.0–8.2, 13 male). No complications were observed during the lavage procedure and no adverse events were noted post-operatively. Total lavage fluid recovered was similar in all groups and the total cell numbers were higher in the VAW group. Eosinophil ($P \leq 0.005$) and mast cell ($P \leq 0.05$) numbers were significantly elevated in the group with atopic asthma.

Conclusions During relatively asymptomatic periods there is on-going airways inflammation, as demonstrated by eosinophil and mast cell recruitment, in children with asthma and atopy but not in children with viral associated wheeze or atopy alone. This strongly suggests that there are different underlying pathophysiological mechanisms in these two groups of children who wheeze.

Keywords: bronchoalveolar lavage, childhood asthma, airways inflammation, eosinophils, mast cells

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Introduction

Airways inflammation has long been recognized as a characteristic pathological feature of severe fatal asthma [1]. More recently, the presence of airways inflammation in adult patients with mild atopic asthma has been recognized [2,3] and it is now thought that the degree of bronchial

hyperactivity is related to the extent of the airways inflammation [4]. Little information is available as to whether these findings also apply to childhood asthma, which may have several different underlying pathophysiological mechanisms. Cutz *et al.* found that the morphological changes in open lung biopsy specimens of two children with well controlled asthma were similar to changes observed at autopsy in two children who died from asthma, although a larger number of submucosal eosinophils and more extensive epithelial damage were noted in the latter patients [5].

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It is probable that atopic children with asthma (atopic asthmatics, AA) and who suffer from frequent interval symptoms will have persisting airways inflammation [6]. However, many children wheeze predominantly in winter and only in association with a viral respiratory infection (viral associated wheeze, VAW) and have completely asymptomatic interval periods [7]. Many of the latter group of children obtain little or no benefit from the regular use of preventative anti-inflammatory therapy [8] and it is possible that these children have a different underlying pathophysiological mechanism that causes them to wheeze only with viral infections.

Fibreoptic bronchoscopy with lavage and biopsy has provided a powerful tool to investigate the airways in adult asthma. In children such procedures present ethical difficulties. We have recently established a method for non-bronchoscopic bronchoalveolar lavage (BAL) in normal children undergoing elective surgery and have obtained normal reference intervals for the cellular data [9]. Using elective surgery as a window of opportunity to overcome ethical difficulties we have now extended this study to investigate the occurrence of persisting airways inflammation in a group of children with a history suggestive of asthma and whose symptoms were relatively quiescent.

Patients and methods

Patients

Children with a history of current asthma (more than two wheezing episodes, with at least one episode having occurred within the previous 12 months) were included. Children with likely alternative causes of wheezing (e.g. resolving bronchopulmonary dysplasia, cystic fibrosis, congenital heart disease or recurrent aspiration) were excluded. All children were free from recent respiratory infections. In addition, children without wheezing but who had evidence of having other atopic illness were recruited to the study. All subjects were to undergo an elective surgical procedure for a non-inflammatory condition at the Royal Belfast Hospital for Sick Children.

From a detailed asthma and allergy history (Table 1) usually taken by one of two physicians (GT, MDS) before the procedure, children were categorized into atopic asthmatics (AA), viral associated wheeze (VAW) and non-asthmatic atopics (NAA). Children whose symptoms were triggered by known aeroallergens, who had other personal atopic features (e.g. atopic eczema, seasonal rhinitis), a strong family background of atopy or an elevated serum IgE compared with age normal values (≥ 2 sd from age normal), were classified as AA. This group of atopic children were further subdivided into those whose asthma symptoms were episodic (i.e. with extended asymptomatic

intervals between asthma attacks) and those with persisting interval symptoms occurring regularly in-between the asthma attacks. Children with a personal history of atopy or elevated IgE but without respiratory involvement were classified as non-asthmatic atopics (NAA). Those children with no personal or strong family background of atopy, whose wheezing was predominantly in winter and occurred solely in association with a viral upper respiratory tract infection were classified as VAW.

Patients were anaesthetized by one of two anaesthetists (RT, TG) using a standard anaesthetic protocol as previously described [9]. Prior to the lavage, children ≤ 5 years were pre-oxygenated for 2–3 breaths. During the surgical procedure, the anaesthetist made minor adjustments to the fractional inspired oxygen concentration according to the oxygen saturation. During the procedure, subsequent surgery and in the early post-operative period heart rate and oxygen saturation were continuously monitored. After intubation, a sterile 8 FG graduated neonatal suction catheter was inserted through the endotracheal tube and wedged in a distal airway. Lavage was performed with a single 20 mL aliquot of normal saline and the lavage fluid was immediately aspirated. Thereafter a blood sample (5 mL) was taken for determination of serum IgE concentrations (commercially available kit, Pharmacia, Sweden). Post-operatively pulse, respiratory rate and temperature were recorded every 30 min for 6 h and thereafter 4 hourly in those staying overnight.

Processing of BAL fluid

Total cell counts were performed using a modified Neubauer haemocytometer (BDH Ltd, UK). Differential cell counting was performed using the glass coverslip method [10] using the modification of Walters and Gardiner [11]. The coverslip preparations were fixed and stained with Diff-Quik® (Baxter Healthcare Ltd, UK) for differential cell counting with at least 500 cells counted per coverslip. Two coverslips were fixed in Carnoy's solution (2 h) followed by staining with toluidine blue (0.5% in 0.5 M HCl, 30 min) for mast cell counts and 2000–5000 cells counted per coverslip. All counts were performed by an observer who was not aware of the subject's clinical condition.

Statistical analysis

Except where otherwise stated data are presented as median and range. Statistical comparisons, where shown, were performed using the Mann–Whitney *U*-test and the Kruskal–Wallis ANOVA test. $P < 0.05$ was considered statistically significant.

Ethical approval

Written informed consent was obtained from the parents of subjects taking part in the study and the study was approved by the Research Ethical Committee of the Faculty of Medicine, The Queen's University of Belfast.

Results

Patient and post-operative data

Ninety-nine children were recruited to the study. Four subjects were excluded from the study. In two cases there was an insufficient lavage return and two samples did not undergo analysis because of red blood cell contamination ($n=1$) and mucous ($n=1$). The patient characteristics are summarized in Table 2.

Plastic surgical procedures were the most common elective operations ($n=59$) and overall the most common procedures were removal of skin or mucous membrane lesions (34), correction of prominent ears (24), urological interventions (17) and tonsils and adenoids (6). The lavage procedure took 1 min. However, in four cases the lavage was repeated as the catheter did not wedge correctly.

No complications were observed during the lavage procedure, although two patients required a slight temporary increase in inspired O_2 concentration immediately post lavage. The anaesthetist did not report any problems caused by the procedure. Heart rate and respiratory rate were recorded post-operatively and no adverse events were noted. It is uncertain whether the mild transient pyrexias observed post-operatively in 11 of the 99 patients were related to the BAL or occurred as a normal post-operative finding. In addition, the majority of children were given routine analgesic medication which also has anti-pyretic actions.

Atopic children with ($n=52$) or without ($n=23$) asthma were significantly older than those with viral associated wheeze ($n=20$) (Table 2). The majority of children in both groups with wheeze were boys.

Lavage data

The lavage fluid recovered was similar in all groups [NAA 30.0% of the infused fluid (range 15.0–42.5); AA 30.0% (10.0–50.0); VAW 32.5% (20.0–45.0)]. The total number of cells recovered for the VAW group [1.36×10^5 cells per mL BAL fluid (0.3–3.43)] was significantly higher than for the NAA ($P=0.0008$) (0.6×10^5 cells per mL BAL fluid (range 0.23–1.43) and for the AA ($P=0.015$) (0.83×10^5 cells per mL BAL fluid (range 0.18–2.50). Differential cell counts are summarized in Table 3. Eosinophils and mast cell percentages were significantly elevated in the group with AA compared with the groups with non-asthmatic atopy or

viral associated wheeze (Table 3, Fig. 1). Since the children with viral associated wheeze were younger than those with atopic asthma, we also compared the differential cell counts for the under-5s in both groups with wheeze (Table 4, Fig. 1). Only the elevation in eosinophil percentages achieved statistical significance in the under 5 year olds with AA compared with the VAW ($P=0.0007$), although mast cell percentages were on average doubled in this group. Using an age-matched sample ($n=16$ each in AA and VAW), again eosinophil ($P=0.016$) but not mast cell percentages were significantly elevated in the AA group (data not shown). All other cell types did not differ significantly. Dividing the children according to sex revealed no statistically significant differences (data not shown).

Dividing the children with atopic asthma into those with persisting interval symptoms ($n=28$) and those with episodic exacerbations without interval symptoms ($n=24$), mast cell and eosinophil numbers were similar in both these groups (Fig. 2). Eosinophils were significantly elevated in children with episodic atopic asthma compared with those with viral associated wheeze ($P=0.006$).

Thirty one atopic asthmatic and 10 children with VAW were currently under treatment with anti-inflammatory anti-asthma medication (inhaled corticosteroids or cromoglycate). When all wheezing children were considered together or atopic asthmatics were considered separately, the use of anti-inflammatory medication was not associated with significant effects on cell differentials (data not shown). However, when the VAW was considered separately, those children on anti-inflammatory medication had reduced mast cell percentages ($P=0.007$) (data not shown).

Discussion

This study clearly demonstrates that even during relatively asymptomatic periods, there is on-going airways inflammation, as demonstrated by the recruitment of eosinophils and mast cells, in children with atopic asthma but not in those children with viral associated wheeze. This strongly suggests that there are different underlying pathophysiological mechanisms in these two groups of children who wheeze. It is possible that adverse airways geometry makes VAW children prone to wheeze with viral infections [12]. In addition, viral and bacterial products are both able to cause mediator release directly and to modulate the response to other secretory stimuli [13,14] and the mast cells or basophils from children with viral associated wheeze may be particularly sensitive to this activation mechanism. Our data provide a possible explanation as to why children with VAW, who do not appear to have chronic airways inflammation, do not respond as well to regular anti-inflammatory treatment [8].

The children were classified according to their clinical

Table 1. Study questionnaire (administered by a doctor)

BRONCHOLAVEOLAR LAVAGE IN CHILDREN

(Study no. _____)

Name: _____ RBHSC#: _____ Date of Study _____

DOB _____ Surgical procedure: _____

Sex _____ Tel no. _____

1. Has your child ever been wheezy Yes/No

2. Has your child ever had a problem with prolonged bouts of coughing Yes/No

3. When was your child last wheezy? _____

At what age did they first start wheezing? _____

4. What makes your child wheeze or cough

a) URTI Yes/No

b) Exercise Yes/No

c) Emotion, e.g. excitement Yes/No

d) Grass or tree pollen Yes/No Specify _____

e) Animals/pets Yes/No Specify _____

Cat _____

Dog _____

Others _____

f) Weather changes Yes/No

g) Which is the worst season? _____

h) Cigarette smoke or other fumes Yes/No

i) Particular location (eg other house) Yes/No

j) Dusting/vacuuming Yes/No

5. Has your child ever been diagnosed as having asthma? Yes/No

When was this diagnosis made? _____

6. How frequently typically does your child wheeze or cough?

Last month

In last 3 months

In last 12 months

No. of asthma attacks

Days/week wheezy or coughing

Nights/week wheezy or coughing

No. hospital admissions for asthma

Days off school for asthma

No. of courses of steroids

Table 1. Continued

7.	Does your child have:	eczema (atopic)	Yes/No
		hay fever	Yes/No
		allergic conjunctivitis	Yes/No
8.	Your child's medication for asthma		
	Drug name	Dose/Freq.	Regular or when required
			How long on this medication
			Drug delivery method
			Problems e.g. with compliance
9.	Your family history		
	Asthma Yes/No	Atopic eczema Yes/No	Other allergies Yes/No eg Hayfever
			Smoker Yes/No
	Father		
	Mother		
	Sibling 1		
	Sibling 2		
	Sibling 3		
10.	CLINICAL EXAMINATION		
	Atopic eczema present:	Yes/No	Comment
	Evidence of allergic rhinitis (eg "allergic salute")	Yes/No	Comment
	Chest shape	Overinflated (barrel)	Yes/No
	Harrison's sulci		Yes/No

Table 2. Characteristics of the three patient groups

	Non-asthmatic atopics (NAA)	Atopic Asthmatics (AA)	Viral associated wheeze (VAW)
Number	23	52	20
Age (years)	8.3 (1.7–13.6)*	8.0 (1.1–15.3)*	3.1 (1.9–8.2)
Sex (M/F)	11/12	36/16	12/8
Present anti-asthma treatment:			
No treatment	23	7	4
Bronchodilators alone		15	6
Disodium cromoglycate (DSCG) alone		4	1
Inhaled corticosteroids (ICS)		26	9
Serum IgE (kU IgE/L)	214 (32– > 1000)	92.3 (3.9– > 1000)	5.0 (< 2–100)

All children receiving inhaled corticosteroids also took bronchodilators on an as required basis. In addition, four of the atopic asthmatic children taking inhaled corticosteroids were additionally receiving salmeterol, three theophylline and one DSCG. One of the VAW children taking inhaled corticosteroids was also receiving DSCG.

All data is presented as absolute number or median (range).

Statistical comparisons were made between the two groups using the Mann–Whitney *U*-test. * = $P < 0.001$, compared to VAW group.

Table 3. Differential cell counts for lavage fluid for atopic asthma and viral associated wheeze patterns of asthma

Cell type (%)	Non-asthmatic atopics (<i>n</i> = 23)		Atopic asthmatics (<i>n</i> = 52)		Viral associated wheeze (<i>n</i> = 20)	
	Median (range)	Mean $\pm$ SEM	Median (range)	Mean $\pm$ SEM	Median (range)	Mean $\pm$ SEM
Macrophage	72.1 (26.5–97.12)	70.4 $\pm$ 4.4	71.3 (25.4–93.8)	69.1 $\pm$ 2.5	64.2 (25.6–91.2)	62.1 $\pm$ 4.7
Lymphocyte	2.2 (0.0–6.0)	2.1 $\pm$ 0.4	1.4 (0.0–10.0)	1.9 $\pm$ 0.3	2.3 (0.1–12.3)	3.4 $\pm$ 0.7
Eosinophil	0.2 (0.0–9.8)**	0.9 $\pm$ 0.4	1.1 (0.0–26.2)	2.5 $\pm$ 0.6	0.2 (0.0–1.1)***	0.2 $\pm$ 0.6
Neutrophil	3.8 (0.4–15.5)	4.7 $\pm$ 0.7	3.5 (0.2–33.2)	8.2 $\pm$ 1.4	9.0 (0.6–69.1)	14.8 $\pm$ 4.1
Epithelial cells	19.3 (1.0–71.8)	21.8 $\pm$ 4.3	14.1 (1.1–56.3)	18.2 $\pm$ 2.0	16.5 (1.6–54.4)	19.4 $\pm$ 3.7
Mast cells	0.14 (0.0–0.49)**	0.18 $\pm$ 0.04	0.33 (0.0–1.15)	0.39 $\pm$ 0.04	0.14 (0.0–0.76)*	0.22 $\pm$ 0.04

* = $P < 0.05$, ** = $P < 0.005$ and *** = $P < 0.0005$ compared to the atopic asthmatic group.

Table 4. Differential cell counts for lavage fluid for atopic asthma and viral associated wheeze patterns of asthma from children under 5 years old

Cell type (%)	Atopic asthmatics (<i>n</i> = 15)		Viral associated wheeze (<i>n</i> = 14)	
	Median (range)	Mean $\pm$ SEM	Median (range)	Mean $\pm$ SEM
Macrophage	67.09 (46.2–90.2)	67.6 $\pm$ 4.1	53.6 (25.6–90.3)	55.1 $\pm$ 5.4
Lymphocyte	1.9 (0.2–4.4)	1.8 $\pm$ 0.3	2.3 (0.1–12.3)	3.3 $\pm$ 0.9
Eosinophil	1.1 (0.1–6.4)*	2.1 $\pm$ 0.6	0.2 (0.0–0.4)	0.2 $\pm$ 0.03
Neutrophil	5.0 (0.4–33.2)	11.4 $\pm$ 3.4	9.0 (0.6–69.1)	17.6 $\pm$ 5.7
Epithelial cells	16.1 (1.1–39.2)	17.1 $\pm$ 3.2	24.1 (2.4–54.4)	23.8 $\pm$ 4.8
Mast cells	0.32 (0.0–1.06)	0.40 $\pm$ 0.10	0.15 (0.02–0.49)	0.20 $\pm$ 0.04

* = $P < 0.001$.

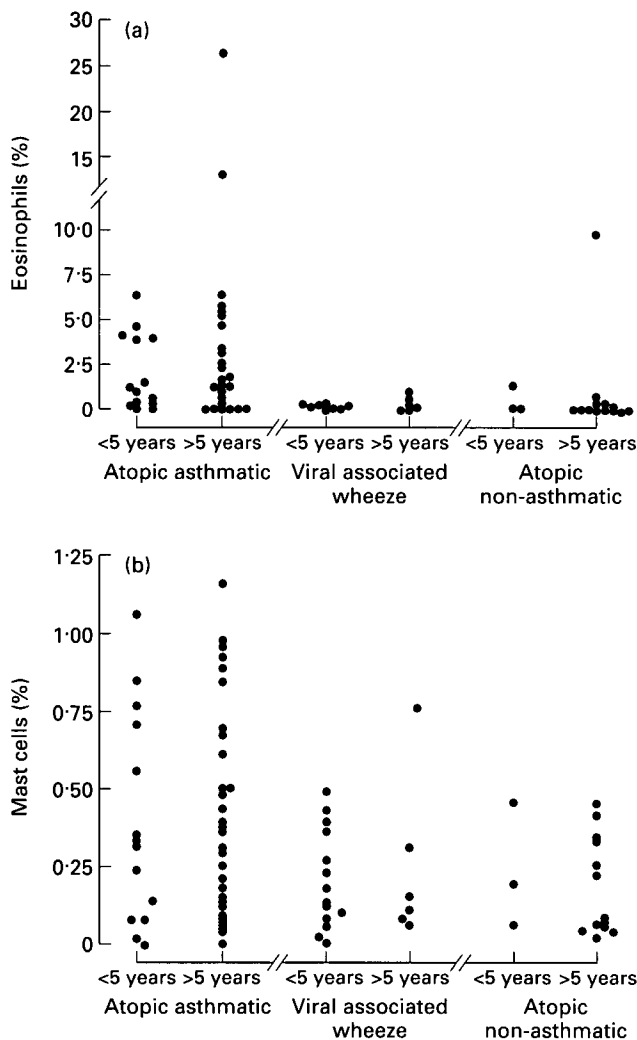


Fig. 1. Eosinophil (a) and mast cell (b) percentages in lavage fluid from children with atopic asthma ($n = 52$), viral associated wheeze ($n = 20$) and atopy without respiratory disease ($n = 23$). The results have been divided according to whether the children were above or below 5 years of age. Over all age groups both eosinophil ($P \leq 0.005$) and mast cell ($P \leq 0.05$) percentages were significantly elevated in the group with atopic asthma compared with the other groups. Considering the under 5-year-old children only, eosinophil numbers were significantly elevated ($P < 0.001$) in the atopic asthma group compared with the group with viral associated wheeze.

history aided by the serum IgE concentration. The serum concentration of IgE is significantly elevated in most patients with allergic diseases such as extrinsic asthma, hay fever and atopic diseases. It is well known that many young children with the clinical pattern of viral associated wheeze will soon outgrow their symptoms, whereas a smaller number go on to develop more classical atopic asthma [15,16]. The differences in eosinophil numbers we found between AA and VAW are unlikely to be due to the

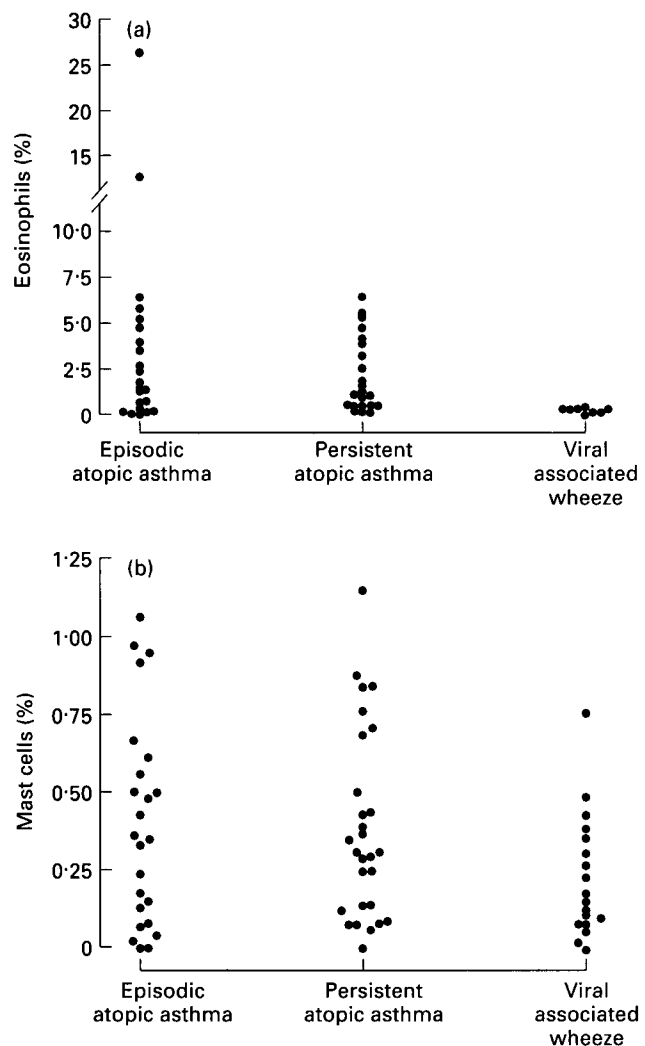


Fig. 2. Eosinophil (a) and mast cell (b) percentages in lavage fluid from children with atopic asthma divided according to whether the symptoms were episodic ($n = 24$) or persistent ($n = 28$). For comparative purposes the data from children with viral associated wheeze has also been included. Eosinophil and mast cell numbers did not differ in the two atopic asthmatic groups. However, eosinophil numbers were significantly elevated ($P < 0.01$) compared with the group with viral associated wheeze.

differences in age as the elevated eosinophil counts were found when children < 5 years were studied or when exactly age-matched subjects were compared. Our data suggests that persistent airways inflammation is related to the presence of atopy. However, some of the children in our VAW group, who were on average younger, may subsequently develop atopy. In contrast, children with atopy but no respiratory disease, did not have increased eosinophils in their lavage fluid. Comparing our data from this study with our previously published results from normal non-atopic children [9]; mast cells and eosinophils were elevated in

the atopic asthmatic but not the viral associated wheeze group.

Both the volume return and total cell counts are in agreement with fiberoptic bronchoscopic investigations on adult asthmatics [17–19] and on normal and asthmatic children [6,9,20–22]. Elevated eosinophil numbers are found in bronchoalveolar lavage fluid from adult atopic asthmatics compared with normals [17–19,23–25]; a finding which is also reported for children with atopic asthma [26–27]. In contrast, we and others have found that these inflammatory cells were not elevated in the lavageate from children who wheeze only with upper respiratory tract infections and who have no evidence of atopy, suggesting that atopy is an important state for the development of persisting airways inflammation in children who wheeze [26–28]. We have found no evidence that airways eosinophils and mast cells are increased in non-asthmatic atopic children. Thus, atopy alone is not causal for this finding. It will be important to follow-up the one non-asthmatic atopic child who had a high BAL eosinophil count to see if he subsequently develops asthma symptoms. A previous study reported increased eosinophils in BAL fluid from older asthmatics/adolescents (8–17 years) but failed to demonstrate mast cells [6]. In our study, we used the glass coverslip technique [10,11] and specialized fixing and staining methods which enabled the enumeration of mast cells. Eosinophils were similarly elevated in atopic asthmatic children under and over 5 years, suggesting that airways inflammation begins at a very early age.

Some children with atopic asthma have episodic asthma exacerbations, whilst others have frequent symptoms persisting in the intervals between asthma attacks. Our data suggest that airways inflammation persists during relatively asymptomatic periods in atopic children with both symptom patterns (Fig. 2).

The immunomodulatory effect of inhaled anti-inflammatory therapy on the airways is well characterized [29]. The majority of the children in our atopic asthmatic group (31 of 52) were taking either inhaled corticosteroids or disodium cromoglycate and despite this therapy we found evidence of airways inflammation, there being no difference between those taking/not taking anti-inflammatory therapy. This study was not designed to determine the effects of this treatment on BAL cell counts. From the clinical information obtained, we considered that some of the children untreated or on bronchodilators alone were in need of regular anti-inflammatory therapy, thus it is therefore not possible to conclude that those children on anti-inflammatory treatment had more severe asthma and that the effect of this medication had been to reduce the cellular counts to similar levels seen in those untreated or only requiring bronchodilators. Furthermore, there was no relationship between the time of the last symptoms and the drug treatment. It is unlikely that

the anti-inflammatory medication used in 10 of the 20 children in the group with viral associated wheeze has caused complete suppression of airways inflammation. In addition, some children from both groups were found to be using inhaled corticosteroids on a 'when required' basis. We have calculated that our study, has a 90% power to detect a 45% reduction in eosinophil numbers between atopic asthmatic children on ($n=31$) and not on ($n=21$) anti-inflammatory therapy. We did not detect any difference. A reduction in eosinophil numbers of 45% would be small when considering the differences in eosinophil numbers when atopic asthmatics are compared with VAW or non-asthmatic atopics (Table 3).

The non-bronchoscopic lavage method used was a modification of the technique described by Koumbourlis and Kurland [31]. We have used this method to quickly and safely obtain bronchoalveolar lavage fluid from asthmatic children attending for routine surgical procedures and it causes little or no interruption to busy surgical lists.

We conclude that during relatively asymptomatic periods on-going airways inflammation occurs in children with atopic asthma but not in those with viral associated wheeze without evidence of atopy. Furthermore, clinical trials investigating new asthma medications for children usually consider all children with wheeze as homogenous and their design should now consider separating patients into the different patterns of childhood asthma.

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