

ORIGINAL ARTICLE

Absence of airway inflammation in a large proportion of adolescents with asthma

COLLIN R. BROOKS,¹ CHRISTINE J. van DALEN,¹ ANGELA ZACHARASIEWICZ,² JODIE L. SIMPSON,^{3,4} JACQUE L. HARPER,⁵ GRAHAM LE GROS,⁵ PETER G. GIBSON,³ NEIL PEARCE^{1,6} AND JEROEN DOUWES¹

¹Centre for Public Health Research, Massey University, and ⁵Malaghan Institute of Medical Research, Wellington, New Zealand, ²Department of Pediatrics and Adolescent Medicine, Wilhelminenspital, Vienna, Austria, ³Respiratory and Sleep Medicine, School of Medicine and Public Health, University of Newcastle, ⁴School of Biomedical Science and Pharmacy, Newcastle, New South Wales, Australia, and ⁶Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom

ABSTRACT

Background and objective: Neutrophilic inflammation has been implicated in non-eosinophilic asthma (NEA) in adults, but little is known about NEA in children/adolescents. We assessed clinical and inflammatory characteristics of NEA in adolescent asthma.

Methods: Airway inflammation, sputum endotoxin, airway hyper-reactivity, atopy and lung function were assessed in 77 adolescents with asthma and 68 without asthma (12–17 years). Asthma was identified on the basis of wheeze and asthma history.

Results: The proportion of NEA (sputum eosinophils <2.5%) was 54%. In this group, atopy, sputum neutrophil, eosinophil, eosinophil cationic protein (ECP), endotoxin, neutrophil elastase and IL-8 levels were not different from those without asthma. In contrast, eosinophilic asthma (EA) was associated with atopy and sputum ECP and IL-8. The majority of NEA had no evidence of inflammation; only 14% had neutrophilia (≥61% neutrophils), compared with 11% of EA, and 15% of those without asthma. Small differences in FEV₁ (NS) were found between EA and NEA, but symptom prevalence and severity was not different (63% of EA and 52% of NEA were classified moderate to severe).

Conclusion: NEA is common in adolescent asthma and has similar clinical characteristics as EA. Neutrophils do not appear to play a role in NEA in adolescents, and underlying mechanisms may not involve airway inflammation.

Key words: adolescent, airway inflammation, asthma, eosinophil, induced sputum.

SUMMARY AT A GLANCE

Our study suggests that many adolescents with stable asthma do not appear to correspond to the conventional notion that asthma is 'an inflammatory disorder of the airways'. In the absence of overt inflammation, the mechanisms responsible for asthma symptoms in a large proportion of adolescent with asthma remain unknown.

Abbreviations: AHR, airway hyper-reactivity; EA, eosinophilic asthma; ECP, eosinophil cationic protein; FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroids; IL, interleukin; ISAAC, International Study of Asthma and Allergies in Childhood; MGA, mixed granulocytic asthma; MMP, matrix metalloprotease; NA, neutrophilic asthma; NE, neutrophil elastase; NEA, non-eosinophilic asthma.

INTRODUCTION

Asthma is often considered an allergic disease involving TH₂-mediated eosinophilic airways inflammation.¹ However, there is increasing evidence that non-allergic mechanisms may also be important. We have previously proposed that 'only' 50% of asthma cases are attributable to eosinophilic airway inflammation, and that non-eosinophilic asthma (NEA) may be due to neutrophilic inflammation.² Other studies also suggest a role for neutrophils in adult NEA as reflected by increased airway neutrophil levels and associated chemokines including interleukin (IL)-8, neutrophil elastase (NE), matrix metalloprotease (MMP)-9,^{3–5} increased gene expression of innate immune receptors and sputum bacterial endotoxin.⁶ However, neutrophilic inflammation in NEA is not always present,⁷ indicating that neutrophils may be important in only a proportion of NEA. In particular, we have previously observed that no overt evidence of either eosinophilic or neutrophilic airway inflammation in over 30% of adults with asthma.³

Correspondence: Jeroen Douwes, Centre for Public Health Research, Massey University, Private Box 756, Wellington, New Zealand. E-mail: J.Douwes@massey.ac.nz

Disclosure Statement: PGG has received honoraria from AstraZeneca, GlaxoSmithKline and Novartis. AZ has received honoraria and travel expenses from Novartis, Chiesi, AOP, AstraZeneca and Forest.

Received 15 March 2015; revised 30 August 2015; accepted 1 September 2015. (Associate Editor: Giorgio Piacentini)

Article first published online: 22 December 2015

Studies in adults have suggested that eosinophilic asthma (EA) is associated with increased severity⁸ and exacerbation frequency⁹ compared with NEA although this is not always observed.^{3,7} Those with NEA also appear less atopic, have normal sub-epithelial layer thickness and poor short-term response to inhaled corticosteroids (ICS).⁵ Thus, EA and NEA may represent distinct phenotypes with potentially different causes and treatment strategies.

There are similar conflicting data in the limited number of studies of NEA in children or adolescents. In particular, EA is associated with reduced lung function,¹⁰ increased airway hyper-reactivity (AHR)¹¹ and uncontrolled asthma¹² in some studies, but other studies find no significant clinical differences between EA and NEA.^{13,14} Equivocal findings have also been reported regarding the importance of neutrophils in childhood NEA.^{14–17}

The aims of this study were to assess: (i) the importance of NEA in adolescents; (ii) the contribution of airway neutrophils, associated inflammatory mediators and bacterial endotoxin (previously associated with neutrophilic inflammation in adults⁶) in adolescent NEA; and (iii) whether EA and NEA in adolescents differ with regard to atopy, lung function, AHR, medication use and severity.

METHODS

Study population

We recruited students aged 12–17 years who previously participated in the ISAAC Phase III survey.¹⁸ Positive respondents to both of the following questions were invited: 'Have you had wheezing or whistling in the chest in the past 12 months?', and 'Have you ever had asthma?' ($n = 244$); random samples without respiratory symptoms were also invited ($n = 595$). Those who agreed to participate (136 with and 134 without symptoms) completed a second questionnaire based on the ISAAC Phase III survey. Approval was obtained from the Wellington Ethics Committee (00/03/010). Written consent was obtained from the participants and their parents.

Asthma definition

Using data from the second questionnaire, we defined asthma based on a positive response to both of the questions above, and/or asthma medication use in the past 12 months. Of the 77 with asthma for whom we had sputum data (see below), 61 had a positive response to all three questions, 71 had a positive response to the wheeze and asthma questions but did not use asthma medication; six used asthma medication and had reported asthma. Those subjects without asthma had no wheezing/whistling in the chest or asthma medication in the last 12 months, no asthma history and no nocturnal cough in the last 12 months apart from that associated with respiratory infection. Sixteen subjects were excluded because of ambiguous questionnaire responses, and eight subjects were unable to be re-contacted.

Classification of asthma severity

Severity was defined by wheezing attack frequency per year (mild intermittent, 0–3; persistent–moderate, 4–12; severe, > 12), sleep disturbance due to wheeze (mild, <1 night/week; moderate, ≥ 1 night/week), daily symptoms in last 2 weeks (moderate), daily use of β_2 agonist (moderate), and forced expiratory volume in 1 s (FEV₁) (mild, $\geq 80\%$ predicted; moderate, $\geq 60\%$ and <80%; severe, <60%) following 2008 Global Initiative for Asthma guidelines (for observational studies rather than clinical management).¹⁹ Asthma severity was assigned according to the highest severity class for any parameter.

Study procedures

All participants underwent a standard clinical assessment. Those who had a respiratory infection within 4 weeks returned at a later date. Atopy was defined as a positive skin prick test to at least one of eight allergens, and lung function was measured following standard procedures (Supplementary Appendix S1).²⁰ Eleven subjects were unable to perform adequate spirometry, and 11 were excluded because of β_2 -agonist use within 6 h prior to assessment.

Combined bronchial provocation and sputum induction using 4.5% saline were conducted as previously described (Supplementary Appendix S1).¹¹ Subjects with sputum eosinophils $\geq 2.5\%$ were classified as EA with the remainder classified as NEA.²¹ Further phenotyping on the basis of sputum neutrophils $\geq 61\%$ was also conducted.³ Seventy-eight (35%) subjects failed to provide an adequate sputum sample; therefore, complete data were available for 77 with asthma and 68 without asthma.

Eosinophil cationic protein (ECP) and IL-8 in sputum supernatant were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). Active NE was measured using *n*-methoxysuccinyl-l-alanyl-l-alanyl-prolyl-l-valyl-p-nitroanilide (Sigma, St Louis, MO, USA). Endotoxin was measured using the *Limulus* Amebocyte Lysate assay (Kinetic QCL; BioWhittaker, Walkersville, MD, USA). These assays have previously been validated for assessment of induced sputum.⁶

Statistical analysis

Lung function was summarized as mean values with 95% CI. Cell counts, inflammatory markers and endotoxin were expressed as median values with 25th/75th percentiles. Chi-square and *t*-tests were performed to test differences in prevalence and mean levels, respectively. The Wilcoxon rank-sum test was used for non-parametric data. Prevalence OR were calculated using logistic regression for analyses involving dichotomous outcomes; linear regression analyses were used for continuous outcomes. The population-attributable fraction was calculated to assess the proportion of asthma attributable to eosinophilia. Data were analysed using SAS (SAS Institute, Cary, NC, USA) and STATA 11 (Statacorp, College Station, TX, USA).

RESULTS

Those with asthma and those without did not differ with regards to age, sex and ethnicity, but those with asthma had, as expected, a small but significantly reduced FEV₁ and FEV₁/FVC (forced vital capacity), and significantly increased prevalence of atopy and AHR (Table 1). Approximately 13% of subjects with asthma were classified as severe, 44% as moderate and 43% as mild.

Fifty-four per cent ($n = 42$) of subjects with asthma had sputum eosinophil levels $<2.5\%$ and were designated NEA, with the remaining 46% designated EA. Eosinophil and ECP levels were significantly higher in EA than NEA or without asthma (Fig. 1a; Table 2), and those with EA were more likely to be atopic (Table 3). Eosinophil levels were similar in NEA and those without asthma (Fig. 1b). Neutrophil levels were not significantly different between those with asthma and those without (Supplementary Fig. S1a), or between EA and NEA (Supplementary Fig. S1b).

Thirty-one subjects with asthma (40%) had EA in the absence of raised neutrophils, and 10 (13%) had sputum neutrophil levels $\geq 61\%$. Of these, 6 (8%) were classified as neutrophilic asthma (NA) and 4 (5%) mixed granulocytic asthma (MGA). The remainder ($n = 36$; 47%) had no evidence of granulocytic inflammation, and were classified as paucigranulocytic asthma. Supplementary Table S1 shows the inflam-

matory and clinical characteristics of those with asthma stratified accordingly. Due to the small numbers of NA and MGA, further analyses were conducted using the NEA and EA classifications only.

Lung function in NEA was marginally (non-significantly) higher than in EA (Table 3). Similarly, AHR, asthma medication use and most symptoms were lower in NEA, but differences were small and not statistically significant. Although EA were slightly more likely to use ICS (Table 3), there were no significant differences in sputum eosinophil percentage when those with asthma were stratified by recent ICS use (Supplementary Fig. S2). The prevalence of moderate-to-severe asthma was similar between both groups (63% and 52% in EA and NEA respectively), with 9% of EA classified as severe compared with 17% of NEA (not significant (NS); Table 3). The EA group was more likely to have persistent asthma, although this was not significant (Table 3). Severity was not associated with eosinophils or neutrophils in all subjects with asthma or EA or NEA respectively (data not

Table 1 Characteristics of study population

	No asthma $n = 68$	Asthma $n = 77$
Age (SD)	15.0 (1.04)	15.1 (1.01)
Male	72.1%	76.6%
Ethnicity		
European	75.0%	68.8%
Non-European	25.0%	31.2%
Lung function		
FVC %-predicted (Mean, SD)	101.1% (12.0)	102.3% (12.8)
FEV ₁ %-predicted (Mean, SD)	100.9% (11.4)	96.4% (11.9)*
FEV ₁ /FVC %-predicted (Mean, SD)	100.2% (7.6)	94.4% (7.9)**
Atopy [†]	36.8%	80.5%**
Airway hyper-reactivity [‡]	7.6%	49.4%**
Asthma severity		
Mild	—	42.9%
Moderate	—	44.2%
Severe	—	13.0%
Asthma persistence		
Intermittent	—	53.2%
Persistent	—	46.8%

* $P < 0.05$; ** $P < 0.01$.

[†]Positive SPT against one or more common allergens.

[‡] $\geq 15\%$ drop in FEV₁ from baseline following a 4.5% saline challenge.

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; SPT, skin prick test.

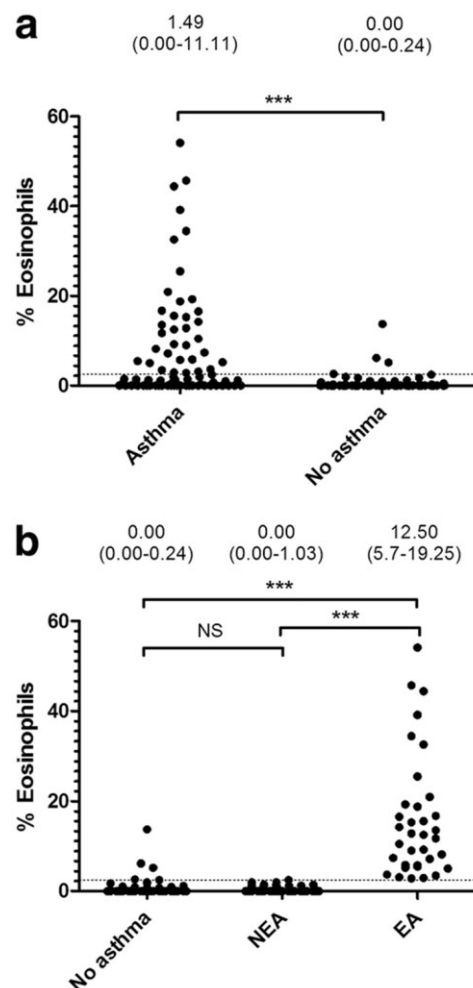


Figure 1 Sputum eosinophil percentages in adolescents with (a) asthma and no asthma and (b) no asthma, non-eosinophilic asthma and eosinophilic asthma (expressed as median (25th–75th percentile)) (***) $P < 0.0001$; NS, not significant).

Table 2 Markers of inflammation and endotoxin levels in sputum

	No asthma <i>n</i> = 68	All asthma <i>n</i> = 77	Eosinophilic asthma <i>n</i> = 35	Non-eosinophilic asthma <i>n</i> = 42
Total eosinophils ($\times 10^4/\text{mL}$)	0.0 (0.0–3.8)	28.1 (0.0–228.7)**	229.9 (89.3–385.7)**	0.0 (0.0–6.8) [†]
Total neutrophils ($\times 10^4/\text{mL}$)	262.4 (49.0–716.0)	216 (40.6–941.8)	242.6 (74.0–1233.7)	170.1 (28.8–856.4)
ECP (ng/mL)	9.0 (2.1–34.3)	44.6 (9.6–193.8)**	168.1 (70.3–231.9)**	12.1 (3.4–49.8) [†]
NE ($\mu\text{g/mL}$) [†]	43.8 (43.8–337.2)	43.8 (43.8–193.8)	43.8 (43.8–43.8)	43.8 (43.8–316.6)
IL-8 (ng/mL)	117.8 (36.1–342.0)	190.5 (52.1–715.9)	295 (94.8–1008.8)*	163 (46.9–466.3)
Endotoxin (EU/mL)	1.4 (0.7–2.8)	1.2 (0.6–3.0)	1.4 (0.6–3.0)	1.1 (0.5–2.3)
Squamous cells (%)	7.6 (4.1–23.0)	13.9 (3.4–26.5)	5.9 (1.4–14.8)	22.9 (6.1–31.2) [†]
Columnar epithelials (%)	0.0 (0.0–0.0)	0.2 (0.0–0.7)**	0.2 (0.0–1.0)	0.2 (0.0–0.6)

Expressed as median (25th–75th percentile).

* $P < 0.05$; ** $P < 0.01$ with asthma versus the reference population.

[†] $P < 0.01$ non-eosinophilic versus eosinophilic asthma.

[†]NE limit of detection = 43.8 $\mu\text{g/mL}$; no asthma, 66% non-detectable; all asthma, 68% non-detectable; EA, 80% non-detectable; NEA, 57% non-detectable.

EA, eosinophilic asthma; NE, neutrophil elastase; NEA, non-eosinophilic asthma.

shown). Despite this, sputum eosinophils were inversely associated with lung function, reaching statistical significance for total eosinophils and FEV₁ %-predicted ($P < 0.05$; Supplementary Table S2).

Mediators associated with granulocytic inflammation (ECP, NE and IL-8) were inversely associated with FEV₁/FVC-%-predicted, but not with other lung function variables (Supplementary Table S2). Although undetectable in 62% ($n = 90$) of samples, NE correlated with neutrophils, but not with eosinophils (Supplementary Table S3). No differences in NE levels were found between EA, NEA and non-asthma subjects, although both NA and MGA were associated with increased NE levels (Supplementary Table S1; NS). However, IL-8 was elevated in EA, but not in NEA (Table 2). Despite this, IL-8 was correlated with both eosinophils and neutrophils. ECP was strongly correlated with eosinophils (Supplementary Table S3), and both were associated with atopy and AHR (Supplementary Table S2). No differences were found in sputum endotoxin levels for any of the studied subgroups (Table 2), and while endotoxin was weakly correlated with NE ($r = 0.17$; $P < 0.05$) (Supplementary Table S3), it was not associated with lung function, atopy or AHR (Supplementary Table S2). Associations were similar when analyses were restricted to only subjects with asthma, but results were generally no longer significant. Adjusting analyses for asthma status, ICS use or using a lower cut-off (54%) to define airway neutrophilia¹² did not significantly alter the results.

DISCUSSION

NEA was slightly more common than EA and those with NEA had marginally higher lung function, fewer symptoms and lower AHR, but differences were not statistically significant. The proportion of moderate-to-severe asthma in both groups was also similar. Neutrophil levels were not increased in NEA, and no significant differences in inflammatory markers were

found between NEA and those without asthma. Thus, despite similar symptoms and clinical characteristics to those observed in EA, there was no evidence of overt airway inflammation in NEA.

Fifty-four per cent of subjects with asthma had no raised sputum eosinophils, and the proportion of asthma attributable to eosinophilia (PAR) was only 43%, which is comparable with previous studies in adults^{2,3} and children.^{22,23} ICS use may have suppressed airway eosinophils and therefore contributed to the low EA prevalence.^{23,24} However, given that 68% of the NEA group had not used ICS in the last 2 weeks, and we saw no difference in sputum eosinophil levels between those who had used ICS in the last 2 weeks and those who had not, ICS use is unlikely to explain these findings. Indeed, this suggests that a substantial proportion of asthma in adolescents is not associated with appreciable airway eosinophilia.

Although neutrophilic bronchitis is associated with viral exacerbations²⁵ and ICS use in adults,²⁴ we found no evidence that neutrophils are involved in stable NEA in adolescents with highly comparable neutrophil levels between NEA, EA and non-asthma subjects. To our knowledge, only two studies have shown evidence of neutrophilic airway inflammation in childhood asthma, but neutrophil percentages (median levels 8.3%¹⁵ and 18%¹⁴) were considerably lower than in healthy reference populations.²⁶ Other studies have also suggested that NA in children is rare.^{16,17} Thus, neutrophilic involvement may be less important in childhood/adolescent asthma compared with adult asthma, where it may be associated with smoking and occupational exposures.

In the absence of granulocytic inflammation, the causes of NEA remain unclear. It is possible that eosinophilic inflammation is present at other airway sites,²⁷ or that the cut-off used for eosinophils ($>2.5\%$)²¹ has misclassified some asthma subjects as NEA. However, this is unlikely given that in 62% of NEA sputum eosinophils were absent. Misclassification of asthma status or inclusion of subjects with asthma in remission may also have

Table 3 Differences in clinical characteristics between eosinophilic and non-eosinophilic asthma subjects

	Eosinophilic asthmatics (EA), <i>n</i> = 35	Non-eosinophilic asthmatics (NEA), <i>n</i> = 42	NEA versus EA <i>n</i> = 77
	Mean (SD)	Mean (SD)	Δ %-predicted (95% CI)
Lung function			
FVC %-predicted	102.0 (14.1)	102.5 (11.8)	0.5 (−5.4–6.4)
FEV ₁ %-predicted	93.8 (10.6)	98.6 (12.5)	4.8 (−0.5–10.1)
FEV ₁ /FVC %-predicted	92.5 (7.3)	96.1 (8.1)	3.6 (0.01–7.1)*
	%	%	OR (95% CI)
Atopy [†]	100.0	64.3	undefined [§]
Airway hyperreactivity [†]	57.1	42.9	0.5 (0.2–1.2)
Asthma medication			
No asthma medication in last 12 months	0.0	23.8	undefined [§]
ICS in the last 12 months	74.3	54.8	0.4 (0.1–1.2)
ICS in the last 2 weeks	48.6	31.0	0.6 (0.2–1.5)
β ₂ -agonist in the last 12 months	100.0	69.1	undefined [§]
β ₂ -agonist ≥ once a day in last 2 weeks	65.7	42.9	0.4 (0.2–1.1)
Wheezing attacks in the last 12 months	%	%	%
0–3 times	60.0	66.7	1.7 (0.6–4.6)
4–12 times	31.4	16.7	0.4 (0.1–1.4)
>12 times	8.6	16.7	1.3 (0.3–6.0)
Sleep disturbance due to wheeze in last 12 months (nights/week)			
Never	54.3	66.7	2.1 (0.8–5.6)
<than once a week	40.0	31.0	0.6 (0.2–1.7)
≥once a week	5.7	2.4	0.4 (0.0–5.2)
Speech limited by wheeze in last 12 months	28.6	19.1	0.5 (0.2–1.6)
Asthma severity			
Mild	37.1	47.6	1.8 (0.7–5.0)
Moderate	54.3	35.7	0.5 (0.2–1.3)
Severe	8.6	16.7	1.3 (0.3–6.0)
Asthma persistence			
Intermittent	48.6	57.1	1.5 (0.6–3.9)
Persistent	51.4	42.9	0.7 (0.3–1.8)

Analyses were adjusted for age, sex and ethnicity.

* $P < 0.05$; ** $P < 0.01$.

[†]Positive SPT against one or more common allergens.

[‡]≥15% drop in FEV₁ from baseline following a 4.5% saline challenge.

[§]OR were undefined because either all or none of the subjects in the EA group were positive for the specific symptom or condition. FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; ICS, inhaled corticosteroids; OR, odds ratio; SPT, skin prick test.

occurred. Indeed, assessing asthma in adolescents is challenging as they often have 'normal' lung function and/or show little response to β-agonists.²⁸ Nonetheless, previous studies have shown that self-reported symptoms compare well with objective markers of asthma,²⁹ and our definition of asthma using the ISAAC questionnaire shows high sensitivity and specificity for physician-diagnosed asthma³⁰ and bronchial hyper-responsiveness.³¹ Also, the prevalence of moderate-to-severe symptoms was similar between EA and NEA. Thus, although misclassification cannot be excluded, it appears unlikely to be a major issue. Our results therefore suggest that in a substantial proportion of adolescent asthma, symptoms may be caused by alternative non-inflammatory mechanisms, which may have neuro-genic or structural origins,³² and which may occur in the absence of ongoing airway inflammation in asthma.^{33,34}

We observed no significant clinical differences between EA and NEA in agreement with previous reports.^{13,14} However, as with adults,^{8,9} there are also reports showing that eosinophilic inflammation is associated with greater severity and lung function deficits in childhood asthma.^{10,12} Furthermore, Lee *et al.* showed that sputum eosinophils or neutrophils in EA or NEA, respectively, were associated with increased severity.²³ In contrast, we found that granulocytes in either EA or NEA were not associated with severity; and-moderate-to-severe symptoms had similar prevalence in both phenotypes. In fact, contrary to some previous reports, severe symptoms were more common in NEA than EA, although this did not reach statistical significance.

We found a significant inverse association between eosinophils and FEV₁ %-predicted, but no associations with other lung function parameters were observed. We also observed a positive (non-

significant) association between neutrophils and FEV₁-%-predicted and FEV₁/FVC-%-predicted. The mechanisms for this are unclear. The lack of a clear association between lung function and airway inflammation may be due to lung function in younger subjects with asthma being less strongly associated with symptoms compared with adults.²⁸

Increased sputum ECP was detected in EA and correlated with eosinophils, as shown previously.²⁸ Similarly, NA and MGA were associated (NS) with increased levels of NE and IL-8 (Supplementary Table S1), as previously described.³ Finally, endotoxin levels did not differ between the groups studied, in contrast with previous findings of increased endotoxin levels in adult NA.⁶ Indeed, sputum endotoxin levels in this study were >100 fold lower than previously reported in adults. The higher levels in adults may represent increased airway bacterial colonization during natural ageing.³⁵

We observed a significantly higher percentage of sputum squamous cells in NEA compared with EA. The basis for this is unclear. It is possible that the reduced inflammatory infiltrate in NEA resulted in squamous cells simply representing a larger percentage of cells overall. In support, we have previously found reduced squamous cells in groups with neutrophilic inflammation.³

A limitation of this study was that only one test per participant was conducted. Some repeated sputum induction studies in adults indicated that NEA is reproducible.^{3,36} In contrast, other studies, including two in children, suggest that inflammatory phenotypes are prone to temporal variability,^{16,17} with some showing a role for ICS treatment in phenotype changes.²⁴ Despite these findings, we observed NEA in patients with and without ICS treatment and eosinophilic airway inflammation can persist in patients with ICS-controlled asthma,¹² suggesting that EA and NEA can occur independent of ICS treatment. Nonetheless, we cannot exclude that temporal changes may have affected our results.

In summary, NEA represented approximately 50% of adolescent asthma and was not associated with neutrophilic inflammation or other markers of inflammation. Therefore, targeting therapeutic interventions solely towards inflammation in NEA may not be effective in a large proportion of adolescents with asthma; indeed, it is possible that absence of inflammation may underlie the poor response to ICS previously observed in >30% of asthma subjects.³⁷ Our study adds to previous data^{3,7} challenging the paradigm that asthma is universally an inflammatory disorder.¹ Further research on mechanisms underlying NEA is necessary to guide the development of more effective therapies (and control measures) for NEA, which makes up a considerable proportion of childhood and adolescent asthma.

Acknowledgements

We thank the study participants, Elizabeth Harding for her involvement with the clinical assessment and hypertonic saline challenge components of the study, and Soo Cheng for her involvement in data analysis. The Centre for Public Health Research is funded by a Programme Grant from the Health

Research Council (HRC) of New Zealand, and this research was funded by an HRC Project Grant.

REFERENCES

- Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat. Rev. Immunol.* 2008; **8**: 183–92.
- Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax* 2002; **57**: 643–8.
- Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006; **11**: 54–61.
- Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. *Chest* 2001; **119**: 1329–36.
- Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002; **57**: 875–9.
- Simpson JL, Grissell TV, Douwes J, Scott RJ, Boyle MJ, Gibson PG. Innate immune activation in neutrophilic asthma and bronchiectasis. *Thorax* 2007; **62**: 211–18.
- Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, Bradding P, Wardlaw AJ, Pavord ID. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax* 2007; **62**: 1043–9.
- Woodruff PG, Khashayar R, Lazarus SC, Janson S, Avila P, Boushey HA, Segal M, Fahy JV. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. *J. Allergy Clin. Immunol.* 2001; **108**: 753–8.
- Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002; **360**: 1715–21.
- Gibson PG, Simpson JL, Hankin R, Powell H, Henry RL. Relationship between induced sputum eosinophils and the clinical pattern of childhood asthma. *Thorax* 2003; **58**: 116–21.
- Gibson PG, Wlodarczyk JW, Hensley MJ, Gleeson M, Henry RL, Cripps AW, Clancy RL. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am. J. Respir. Crit. Care Med.* 1998; **158**: 36–41.
- Cai Y, Carty K, Henry RL, Gibson PG. Persistence of sputum eosinophilia in children with controlled asthma when compared with healthy children. *Eur. Respir. J.* 1998; **11**: 848–53.
- Baraldo S, Turato G, Bazzan E, Ballarin A, Damin M, Balestro E, Lokar Oliani K, Calabrese F, Maestrelli P, Snijders D *et al.* Noneosinophilic asthma in children: relation with airway remodelling. *Eur. Respir. J.* 2011; **38**: 575–83.
- Drews AC, Pizzichini MM, Pizzichini E, Pereira MU, Pitrez PM, Jones MH, Sly PD, Stein RT. Neutrophilic airway inflammation is a main feature of induced sputum in nonatopic asthmatic children. *Allergy* 2009; **64**: 1597–601.
- Li AM, Tsang TW, Chan DF, Lam HS, So HK, Sung RY, Fok TF. Cough frequency in children with mild asthma correlates with sputum neutrophil count. *Thorax* 2006; **61**: 747–50.
- Wang F, He XY, Baines KJ, Gunawardhana LP, Simpson JL, Li F, Gibson PG. Different inflammatory phenotypes in adults and children with acute asthma. *Eur. Respir. J.* 2011; **38**: 567–74.
- Fleming L, Tsartsali L, Wilson N, Regamey N, Bush A. Sputum inflammatory phenotypes are not stable in children with asthma. *Thorax* 2012; **67**: 675–81.
- Ellwood P, Asher MI, Beasley R, Clayton TO, Stewart AW. The International Study of Asthma and Allergies In Childhood (ISAAC): phase three rationale and methods. *Int. J. Tuberc. Lung Dis.* 2005; **9**: 10–16.

- 19 Global Initiative for Asthma (GINA). *Global Strategy for Asthma Management and Prevention 2008 (Update)*. NHLBI/WHO Workshop Report. *Global Initiatives for Asthma*. NHLBI, Washington, DC, 2008.
- 20 American Thoracic Society (ATS). Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am. J. Respir. Crit. Care Med.* 1995; **152**: 1107–36.
- 21 Gibson PG, Henry RL, Thomas P. Noninvasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate. *Eur. Respir. J.* 2000; **16**: 1008–15.
- 22 Lovett CJ, Whitehead BF, Gibson PG. Eosinophilic airway inflammation and the prognosis of childhood asthma. *Clin. Exp. Allergy* 2007; **37**: 1594–601.
- 23 Lee YJ, Kim KW, Choi BS, Sohn MH, Kim KE. Clinical characteristics of eosinophilic and noneosinophilic asthma in children. *Acta Paediatr.* 2013; **102**: 53–7.
- 24 Hancox RJ, Cowan DC, Aldridge RE, Cowan JO, Palmay R, Williamson A, Town GI, Taylor DR. Asthma phenotypes: consistency of classification using induced sputum. *Respirology* 2012; **17**: 461–6.
- 25 Wark PA, Johnston SL, Moric I, Simpson JL, Hensley MJ, Gibson PG. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. *Eur. Respir. J.* 2002; **19**: 68–75.
- 26 Balbi B, Pignatti P, Corradi M, Baiardi P, Bianchi L, Brunetti G, Radaeli A, Moscato G, Mutti A, Spanevello A *et al.* Bronchoalveolar lavage, sputum and exhaled clinically relevant inflammatory markers: values in healthy adults. *Eur. Respir. J.* 2007; **30**: 769–81.
- 27 Lex C, Ferreira F, Zacharasiewicz A, Nicholson AG, Haslam PL, Wilson NM, Hansel TT, Payne DN, Bush A. Airway eosinophilia in children with severe asthma: predictive values of noninvasive tests. *Am. J. Respir. Crit. Care Med.* 2006; **174**: 1286–91.
- 28 Papadopoulou E, Tzanakis N, Tsoumakidou M, Kyriakoy D, Plataki M, Mantzouranis EC, Siafakas NM. Comparison of induced sputum inflammatory profiles between childhood and adult-onset asthma. *Respir. Med.* 2006; **100**: 1442–50.
- 29 Pekkanen J, Pearce N. Defining asthma in epidemiological studies. *Eur. Respir. J.* 1999; **14**: 951–7.
- 30 Yeatts K, Davis KJ, Sotir M, Herget C, Shy C. Who gets diagnosed with asthma? Frequent wheeze among adolescents with and without a diagnosis of asthma. *Pediatrics* 2003; **111**: 1046–54.
- 31 Jenkins MA, Clarke JR, Carlin JB, Robertson CF, Hopper JL, Dalton MF, Holst DP, Choi K, Giles GG. Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician assessment in the diagnosis of asthma. *Int. J. Epidemiol.* 1996; **25**: 609–16.
- 32 Baroffio M, Barisione G, Crimi E, Brusasco V. Noninflammatory mechanisms of airway hyper-responsiveness in bronchial asthma: an overview. *Ther. Adv. Respir. Dis.* 2009; **3**: 163–74.
- 33 Grainge CL, Lau LC, Ward JA, Dulay V, Lahiff G, Wilson S, Holgate S, Davies DE, Howarth PH. Effect of bronchoconstriction on airway remodeling in asthma. *N. Engl. J. Med.* 2011; **364**: 2006–15.
- 34 Miller BD, Wood BL, Lim J, Ballow M, Hsu C. Depressed children with asthma evidence increased airway resistance: 'vagal bias' as a mechanism? *J. Allergy Clin. Immunol.* 2009; **124**: 66–73 e1–10.
- 35 Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM. Aging of the innate immune system. *Curr. Op. Immunol.* 2010; **22**: 507–13.
- 36 Simpson JL, McElduff P, Gibson PG. Assessment and reproducibility of non-eosinophilic asthma using induced sputum. *Respiration* 2010; **79**: 147–51.
- 37 Szeffler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, Craig TJ, Dolovich M, Drazen JM, Fagan JK *et al.* Significant variability in response to inhaled corticosteroids for persistent asthma. *J. Allergy Clin. Immunol.* 2002; **109**: 410–18.

Supplementary Information

Additional Supplementary Information can be accessed via the *html* version of this article at the publisher's website.

Appendix S1 Methods

Figure S1 Sputum neutrophil percentages in (a) asthma and non-asthma adolescents and (b) non-asthma, non-eosinophilic asthma and eosinophilic asthma (expressed as median (25th–75th centile)) (NS, not significant)

Figure S2 Sputum eosinophil percentages in adolescents with asthma when stratified on the basis of inhaled corticosteroid use in (a) the previous 2 weeks or (b) the previous year (expressed as median (25–75th centile))

Table S1 Clinical characteristics and sputum markers of inflammation in the four inflammatory phenotypes previously described.³ * $P < 0.05$; ** $P < 0.01$ EA versus PGA (n too small for NA and MGA). #Neutrophil elastase limit of detection = 43.8 µg/mL; EA, 90% non-detectable; NA, 50% non-detectable; MGA, 0% non-detectable; PGA, 58% non-detectable.

Table S2 Associations between cell, cytokine and endotoxin levels in sputum and clinical characteristics. Analyses were adjusted for age, sex and ethnicity.

Table S3 Correlations between cell, cytokine, and LPS levels in sputum. * $P < 0.05$; ** $P < 0.01$.