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Exacerbations of asthma without sputum eosinophilia

M O Turner, P Hussack, M R Sears, J Dolovich, F E Hargreave

Abstract

***Background* – Sputum analysis provides a non-invasive method of examining the airway secretions of subjects with asthma in order to better understand the inflammatory process. Increased proportions of eosinophils are generally seen in the sputum of subjects with asthma, especially when there is an exacerbation. An unexpected observation in the sputum of subjects with mild exacerbations of asthma is reported.**

***Methods* – Thirty four consecutive subjects with symptoms consistent with a mild exacerbation of asthma were recruited for a treatment study. Inclusion criteria required persistent symptoms of chest tightness, dyspnoea, or wheezing for two weeks (without spontaneous improvement or alteration in dose of inhaled corticosteroid) and a forced expiratory volume in one second (FEV_1) that was reversible to more than 75% predicted or known best to ensure the exacerbation was mild. Sputum (spontaneous or induced with hypertonic saline) from all subjects was examined for differential cell counts. Eosinophilic sputum was defined as $\geqslant$4% eosinophils on two occasions or >10% eosinophils once. Clinical characteristics, sputum differential counts, and measurements of airways obstruction were compared between the subjects with and without sputum eosinophilia.**

***Results* – Almost half of the subjects (16 of 34) considered to have mildly uncontrolled asthma had no sputum eosinophilia. In comparison with the subjects who had sputum eosinophilia the non-eosinophilic group had less airways obstruction (FEV_1% predicted 88% *v* 70%) and less severe airways hyperresponsiveness (PC_{20} methacholine 0·45 mg/ml *v* 0·13 mg/ml). There was no difference between the groups in the type or prevalence of symptoms, history of recent infections, smoking, relevant allergen exposure, or use of inhaled corticosteroid.**

***Conclusions* – Symptoms of mildly uncontrolled asthma are not always associated with eosinophilic airways inflammation as measured by sputum analysis. The causes and treatment of the non-eosinophilic condition require further investigation.**

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Keywords: asthma, sputum, eosinophils.

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The presence of an eosinophilic cellular infiltrate is regarded as characteristic of airways inflammation in asthma and has been observed in the sputum from natural exacerbations[1] and in exacerbations induced by inhaled allergen,[2 3] inhaled isocyanate,[4] or reduction of steroid treatment.[5] Sputum examination is a non-invasive method of evaluating airways inflammation.[6 7] It is likely that its more widespread use will lead to new observations. A finding previously reported was sputum eosinophilia without the abnormalities of airway function characteristic of asthma.[8 9] The patients were considered to have "eosinophilic bronchitis" and they responded to corticosteroid treatment. In this report we describe an unexpected finding of exacerbations of symptoms in patients without sputum eosinophilia.

Methods

SUBJECTS

Thirty four consecutive adults with asthma whose symptoms of chest tightness, wheezing, or dyspnoea had been worse for at least two weeks were screened for a placebo controlled study comparing the effect of salmeterol and beclomethasone on spirometric parameters and indices of inflammation in sputum and blood (table 1). Subjects were included if symptoms were not improving spontaneously, and were worse than when the asthma was considered to be controlled. The evidence for asthma was either airways responsiveness to methacholine (PC_{20}<8 mg/ml) in subjects with a forced expiratory volume in one second (FEV_1) of $\geqslant$70% predicted (n=23) or, in those with a baseline FEV_1 of <70%, an improvement in FEV_1 of $\geqslant$15% after 200 μg salbutamol (n=11). The asthma exacerbation was considered to be mild as the FEV_1 after salbutamol was only mildly abnormal; in the more severely obstructed subjects (baseline FEV_1 <70% predicted) the FEV_1 increased to $\geqslant$75% predicted or known best. Subjects who did not meet the reversibility criteria or who were using >1000 μg/day inhaled corticosteroid were excluded. There was no evidence of other chronic respiratory disease or purulent sputum and none of the subjects had received treatment with antibiotics or prednisone within the previous month. Thirteen subjects were using low or moderate dose inhaled corticosteroid ($\leqslant$1000 μg/day) but the dose had not changed for at least two weeks. Twenty eight subjects (82%) used an inhaled bronchodilator for symptom relief; the remainder had either elec-

Table 1 Characteristics of subjects with eosinophilic and non-eosinophilic sputum

Subjects	Age (y)	Sex	Asthma duration (y)	FEV_1 (% pred)	FEV_1 (% increase post BA)	PC_{20} (mg/ml)	ICS (μg/day)	BA (μg/day)	Allergen exposed	Smokers	RTI (months ago)	Sputum (I/S)	Sputum eosinophils (%)	Sputum neutrophils (%)
Eosinophilic sputum:														
1	28	M	25	82	15	0·21	400	800	−	−	3·5	I	10·8	50·1
2	59	F	3	70	22	ND	800	1000	−	−	2	S	79·2	10·6
3	19	M	19	74	ND	0·03	250	200	−	−	1·5	I	5·6	20·2
4	42	F	23	75	17	1·9	200	400	−	−	3	I	10·7	71·1
5	38	F	38	82	15	0·33	0	400	+	+	3	I	15·5	40·6
6	20	M	9	77	ND	0·11	0	200	+	−	1	I	25·8	24·1
7	28	M	26	70	ND	0·03	0	500	+	−	5	S	37·8	29·7
8	20	F	20	70	ND	0·35	400	800	+	−	1	I	4·5	27·9
9	50	F	15	64	35	ND	400	1500	+	−	1·25	S	61·3	26·5
10	46	M	34	64	28	0·13	0	1000	+	−	8	I	4·3	87·4
11	47	F	26	45	66	ND	0	1600	−	−	2	I	49·1	10·1
12	65	M	12	69	15	ND	200	200	−	−	12	S	20·1	67·6
13	31	F	7	74	ND	0·04	200	800	+	−	4	S	79·3	15·2
14	26	M	16	71	23	ND	0	500	+	+	12	S	60·4	9·2
15	60	M	20	69	16	ND	0	0	−	−	2	S	72·2	23·3
16	22	F	20	67	36	ND	0	1200	−	−	2	I	46·5	19·8
17	32	M	12	55	28	ND	0	400	−	+	12	S	57·9	17·9
18	24	F	13	82	14	0·08	0	200	+	−	0·75	I	46·0	41·0
Mean	36·5		19	70*	25	0·13	158	650			4·2		38·2*	32·9
SD	15		9	9·3	14		224	467			3·9		26·6	22·8
Non-eosinophilic sputum:														
19	58	F	5	56	15	ND	0	100	−	−	4·0	S	3·1	32·0
20	32	M	24	87	ND	0·54	1000	2000	+	+	2·0	I	2·0	48·7
21	18	F	9	121	2	5·00	500	100	+	−	4·0	I	1·6	11·3
22	21	F	20	82	16	0·22	0	200	−	−	NA	I	1·4	53·8
23	19	F	2	86	ND	0·15	0	300	−	+	4·0	S	2·0	26·0
24	38	M	2	82	23	0·23	0	0	−	+	24·0	I	2·6	41·9
25	21	F	16	105	8	1·25	0	400	+	−	NA	I	2·5	9·7
26	23	F	11	103	ND	5·18	0	600	+	+	2·0	I	1·8	10·3
27	50	M	1	57	22	ND	1000	600	−	−	NA	I	2·6	70·1
28	27	M	24	89	5	0·05	0	200	+	−	0·5	I	1·1	88·4
29	27	F	1	104	ND	0·91	0	0	−	−	NA	I	3·4	11·8
30	22	F	1	84	ND	0·04	0	0	−	−	0·25	S	0·9	71·5
31	53	F	1	90	ND	0·04	800	1000	−	−	3·0	S	3·7	82·5
32	24	M	22	85	ND	0·57	0	0	+	−	5·0	I	2·4	16·9
33	22	F	18	91	ND	3·90	1000	600	+	−	0·5	I	1·3	26·7
34	34	M	1	90	ND	0·87	0	0	−	−	2·0	I	1·0	81·7
Mean	30·6		10	88	13	0·45	269	381			4·3		2·1	43·0
SD	12·8		9	16	8		427	523			6·4		0·9	28·6

FEV_1 = forced expiratory volume in one second; PC_{20} = concentration of methacholine provoking a 20% fall in FEV_1; ICS = inhaled corticosteroid; BA = inhaled β_2 agonist; RTI = history of respiratory tract infection (months ago); I = induced sputum; S = spontaneous sputum; ND = not done; NA = not available.
FEV_1 predicted values from Crapo *et al.*[26]
* Comparisons between groups were significant for FEV_1% pred ($p = 0·01$) and % eosinophils ($p = 0·00001$).

ted not to use their bronchodilator or had not been prescribed a bronchodilator before the screening visit. The study was approved by the hospital research committee and all subjects gave written informed consent.

STUDY DESIGN

Subject characteristics were documented by a structured questionnaire. Spirometric measurements were performed using a nine litre Collins water sealed spirometer. Methacholine airways responsiveness was measured by the method described by Juniper *et al.*[10] Atopic status was determined by skin prick tests with 12 common allergen extracts. Sputum was obtained spontaneously if possible or was induced by inhalation of hypertonic saline aerosol as described by Pin *et al.*[11] It was processed within two hours as described by Popov *et al.*[7] Differential cell counts were obtained from a Wright stained cytospin by a technician blinded to the clinical details.

Sputum was considered to be eosinophilic if the eosinophil differential cell count was $\geqslant 4\%$. An initial eosinophil count of between 4% and 10% in three subjects (4·1%, 4·8%, 4·1%) was confirmed by analysis of a second sputum sample within 21 days (5·6%, 4·5%, 4·3%, respectively) before enrollment in the study. One subject was reclassified to the non-eosinophilic group because the initial eosinophil count of 5·3% was 1·1% on the second occasion. The selection of $\geqslant 4\%$ eosinophils to define eosinophilic sputum was based on the 95% confidence intervals of 0·6% to 3·2% about the mean of 1·9% eosinophils in the sputum smears of 14 normal subjects studied by Pin *et al.*[11] In a subsequent examination of 20 specimens from 10 healthy non-asthmatic subjects using cytospins the mean differential eosinophil count was 0·5% and the 95% confidence interval was 0·3% to 0·7%.[12]

DATA ANALYSIS

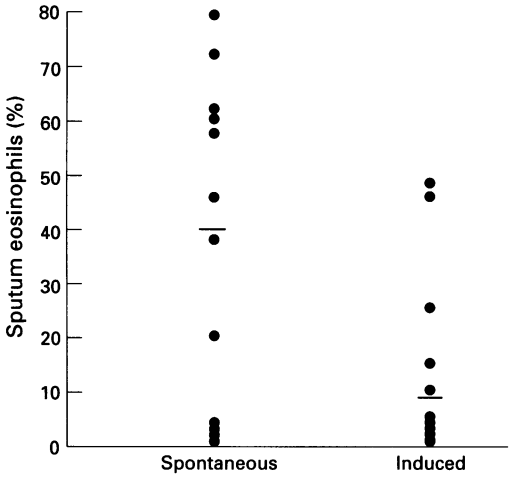
Group characteristics were expressed as mean (SD) or geometric mean (for PC_{20}). Group means were compared by unpaired t tests for continuous variables and by χ^2 for categorical variables. PC_{20} values were logarithmically transformed for analysis. Least squares regression analysis was used to look at the relationship between eosinophil counts, FEV_1, and PC_{20}.

Results

A sputum differential eosinophil count of $\geqslant 4\%$ was found in only 18 of the 34 subjects despite the fact that their current symptoms suggested exacerbated asthma (tables 1 and 2). The total and differential cell counts were otherwise similar between these subjects and those with non-eosinophilic sputum. Subjects who were able to produce spontaneous sputum (eight eos-

Table 2 Mean (SD) sputum total and differential cell counts

	Eosinophilic	*Non-eosinophilic*	*p value*
Total cell count (10^6/ml)	1·5 (2·0)	3·9 (5·4)	
Differential cell count (%)			
Eosinophils	38·2 (26·6)	2·1 (0·9)	0·00001
Neutrophils	32·9 (22·8)	43·0 (28·6)	
Macrophages	22·4 (17·7)	45·9 (29·8)	0·01
Lymphocytes	4·2 (3·5)	3·1 (1·9)	
Bronchial epithelial	2·2 (2·4)	2·8 (5·2)	



Differential cell count of eosinophils in subjects with asthma with spontaneous (n=12) and induced (n=22) sputum. The solid bar represents the mean count for each group. Eosinophil counts were higher in asthmatic subjects who could expectorate sputum spontaneously.

inophilic, four non-eosinophilic) had significantly higher sputum eosinophil counts (mean 40·3%) than those in whom sputum needed to be induced (mean 9·4%, p=0·01) (figure).

The clinical characteristics of the groups with and without sputum eosinophilia were compared (table 1). In the eosinophilic group the prevalence of chest tightness (97% *v* 85%), wheezing (94% *v* 71%), dyspnoea (88% *v* 85%), and cough (88% *v* 71%) was not significantly different from the non-eosinophilic group. However, airways obstruction was significantly more severe (FEV_1% pred 70% *v* 88%, p=0·01) in the eosinophilic subjects. There was a trend only to a greater degree of hyperresponsiveness (PC_{20} methacholine 0·13 mg/ml *v* 0·45 mg/ml) and to more bronchodilator use (94% *v* 69%). Overall, there was a weak correlation between sputum eosinophils and baseline FEV_1($r=-0·50$, p=0·002) and PC_{20}($r=-0·40$, p=0·05).

Possible causes for differences in the sputum eosinophil counts between the eosinophilic and non-eosinophilic group were examined. All but one subject were atopic. Sixteen subjects (nine eosinophilic, seven non-eosinophilic) with positive skin prick tests to seasonal aeroallergens or pets were seen during a period of exposure. The sputum eosinophil counts did not differ between subjects exposed to allergens (21·7%) and all others (20·2%). Furthermore, the proportion of subjects with a history of respiratory tract infection within two months (50% eosinophilic *v* 54% non-eosinophilic), who were smokers (17% *v* 25%), or who were on treatment with inhaled corticosteroid (44% *v* 31%) was not different between the groups.

Discussion

In the course of recruiting subjects for a clinical trial to investigate the anti-inflammatory properties of asthma medications we selected subjects with mild exacerbations of asthma which appeared to be established and not improving. We expected this clinical presentation to be associated with eosinophilic airways inflammation. To our surprise, almost half (47%) of the first 34 consecutive subjects who met the clinical trial entry criteria did not have sputum eosinophilia. The subjects with non-eosinophilic sputum had a trend to less cough, wheeze, and spontaneous sputum production. They had significantly less airways obstruction and there was a trend to fewer bronchodilators and less severe airways hyperresponsiveness than those with eosinophilic sputum. There was no obvious difference in the cause of the exacerbation between the groups. The results raise the possibility that, if the sputum cell counts are reliable, there may be a diversity of causes or a diversity of inflammatory responses in mild, naturally occurring exacerbations of asthma.

The accuracy of the sputum cell counts is suggested by their repeatability. We have shown that differential counts examined on stained smears of sputum (the mean counts of four smears within and between specimens) is highly reproducible.[1 11] In the present study we have replaced sputum smears with stained cytospins made from sputum treated with dithiothreitol to disperse the cells.[7] The cells are easier to recognise and count on cytospins and repeatability of counts on a single cytospin is better than on a single smear. We therefore believe that cytospin counts are a more accurate reflection of the cells found in sputum. In comparison with the smear method, cytospins from healthy subjects show similar eosinophil counts but higher neutrophil counts.[11 12]

In the present study, subjects with spontaneous sputum had higher differential eosinophil counts than those in whom the sputum had to be induced. This raises the possibility that induced sputum underestimates the proportion of eosinophils. However, early comparison of spontaneous and induced sputum in the same subjects has shown similar differential cell counts.[13] The higher eosinophil counts in spontaneous sputum observed in this study are therefore probably the result of more inflammation and secretions and not a failure of induced sputum to provide representative counts.

The ability of examination of the sputum to represent airway mucosal inflammation has been suggested by the responsiveness and validity of the measurements and the comparison with cell counts in bronchial washings and bronchoalveolar lavage (BAL) fluid. The responsiveness has been shown by increases in the proportion of eosinophils in sputum after allergen inhalation causing dual asthmatic re-

sponses and heightened methacholine responsiveness,[2] and by a decrease in eosinophils after corticosteroid treatment.[5 9] The validity of counts has been shown by differences between healthy subjects and two groups who have airways inflammation of different pathogenesis, specifically asthmatics and smokers with non-obstructive bronchitis.[1 11 12 14] Early comparison of cell counts in sputum compared with bronchial washings and BAL fluid have shown that the proportion of eosinophils is highest in sputum.[15] We therefore believe that sputum cell counts in asthma reflect events in the mucosa of the airways.

Subjects in this study all had asthma defined by physiological criteria. With an established exacerbation of symptoms we expected sputum eosinophilia to be present. Why then did almost half of the subjects not have this feature? One possibility is that the sensitivity of sputum eosinophilia to detect a mild exacerbation of asthma defined by symptoms and reversible airways obstruction is poor. The exacerbation was objectively less severe in the non-eosinophilic group. However, sputum eosinophils increase readily after a mild exacerbation of asthma caused by allergen inhalation[2 3] or by a reduction in inhaled steroid treatment.[5] Alternatively, the definition of eosinophilia of $\geqslant 4\%$ may have been set too high. While the level we selected seemed appropriate,[11 12] it is possible that counts below 4% could be raised if baseline sputum eosinophil cell counts in the non-exacerbated state were virtually zero. The answer to these questions will require studies involving a comparison of sputum findings with those in bronchial biopsy samples, and the addition of inhaled steroid treatment to both non-eosinophilic and eosinophilic groups of subjects.

Assuming that the methods of examination of sputum cell counts are reliable, the results raise the possibility of different causes of inflammatory responses. We could not identify obvious differences between the groups of subjects in current exposure to allergens, smoking, and use of inhaled corticosteroids or evidence of infection. Perhaps the symptoms in the non-eosinophilic group were of non-inflammatory origin. Symptoms are known to be non-specific and might be manifestations of stress, hyperventilation, laryngeal dysfunction or increased exposure to causes of bronchoconstriction not associated with inflammatory cell infiltration.

On the other hand, while there was no significant difference between the groups in sputum total cell count or differential neutrophil counts, the neutrophil counts were increased in certain individual subjects. Increased neutrophils in sputum have been reported in a study of more severe exacerbations of asthma,[16] in BAL fluid in non-asthmatic subjects three hours after exposure to ozone,[17] and in the cartilaginous airways of three subjects with sudden onset (less than 1·5 hours) fatal asthma.[18] An increase in neutrophils in bronchial mucus also occurs with infections and asthma exacerbations are commonly associated with viral infection.[19] In future studies the aetiology and types of non-allergic inflammation – for example, infection – need prospective evaluation with objective measurements.

The results of this study illustrate that symptoms of asthma do not necessarily predict eosinophilic airways inflammation in asthma. This should not be a surprise, since accurate assessment of other characteristics of asthma such as airways obstruction,[20] variable airways obstruction,[21] and airways hyperresponsiveness[22] also require direct objective measurements. The results underline the need to measure indices of airways inflammation in asthma and other airways diseases to better understand their causes, clinical effects, and responses to treatment. There is increasing evidence that examination of sputum cell counts is a practical method of measuring airways inflammation in asthma. Sputum measurements in future studies of clinical asthma might also include cellular activation markers[23 24] and fluid phase constituents such as ECP and albumin.[12 14 25]

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