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Role of sputum differential cell count in detecting airway inflammation in patients with chronic bronchial asthma or COPD

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Abstract

***Background* – Sputum may provide an alternative source of bronchial cells to investigate characteristics of airway inflammation and its functional correlates in patients with asthma or chronic obstructive pulmonary disease (COPD).**

***Methods* – Two groups of clinically stable patients were studied: a group of 43 patients with mild or moderate asthma and a group of 18 patients with COPD. Twenty normal subjects formed a control group. Sputum production was either spontaneous or induced with inhaled hypertonic saline for five minute periods for up to 20 minutes. The concentration of saline was increased at intervals of 10 minutes from 3% to 4%. Plugs from the lower respiratory tract were selected for differential counting in cytocentrifugation preparations. Bronchial provocation tests were performed by inhaling progressive concentrations of histamine from a DeVilbiss 646 nebuliser and the concentration of histamine which caused a 20% fall in the forced expiratory volume in one second (FEV_1) was calculated ($PC_{20}FEV_1$).**

***Results* – Neutrophils predominated in the sputum of subjects with COPD while eosinophils predominated in the sputum of those with chronic asthma. However, in 28% of asthmatic subjects an increased percentage of neutrophils was found. In asthmatic patients the differential count of eosinophils was inversely related to the FEV_1, FEV_1/VC, and bronchial hyperresponsiveness, and directly related to clinical scores.**

***Conclusions* – The cellular profile of sputum in normal subjects and in patients with asthma and COPD is different. The concentration of eosinophils in the sputum correlates with the severity of asthma.**

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Airway inflammation plays an important part in the pathogenesis of asthma[1–8] and chronic obstructive pulmonary disease (COPD).[5 7–11] The cellular inflammatory response of the bronchial mucosa is characterised by infiltration of eosinophils and mast cells in asthma,[3–6 8] while infiltration of macrophages, neutrophils, T lymphocytes but not eosinophils is thought to be the predominant pattern in COPD.[5–8 10 12] Recent studies[13 14] have also shown neutrophil inflammation in sputum from asthmatic subjects during exacerbations.

Use of induced sputum cell counts[15–17] has recently been proposed as a non-invasive alternative to bronchoalveolar lavage (BAL) to investigate airway inflammation in asthma and COPD. Sputum examination may also be used to differentiate the inflammatory characteristics of asthma from those of other airway diseases. Inflammatory changes in sputum have been reported to correlate with disease severity[15 16] in asthma. The present study was carried out in an attempt to investigate the cell profile in the sputum of patients with asthma and COPD and to investigate its relation to airflow obstruction.

Methods

SUBJECTS

Forty three patients with chronic bronchial asthma, 18 with chronic obstructive pulmonary disease (COPD), and 20 normal subjects participated in the study. Informed consent was given by each patient and the study was approved by the local ethics committee.

The normal subjects were an age matched group of 20 healthy, lifelong non-smoking, non-allergic subjects aged 20–65 years without symptoms of asthma and normal bronchial responsiveness to histamine (concentration causing a 20% fall in forced expiratory volume in one second > 16 mg/ml). Their pulmonary function was within the normal range (FEV_1 > 80% predicted, FEV_1/VC > 70%).

Asthma was characterised by episodes of dyspnoea with wheezing, variable airflow limitation with reversible obstruction ($\geq$ 20% increase in FEV_1 after inhalation of 200 μg fenoterol), and a positive response to inhalation challenge with histamine. Asthma was classified as mild (24 patients) or moderate (19 patients), according to the criteria of the NHLBI.[18] The clinical severity of asthma was graded from 1 to 5 according to the Aas' scoring system.[19] Before entering the study 12 patients had been given inhaled bronchodilators on demand, 16 required regular inhaled bronchodilator therapy, 15 received daily inhaled corticosteroids, and 11 received daily oral corticosteroids. Six patients were on no drugs. All bronchodilators were withheld for at least 12 hours before each study. All subjects had been free from acute respiratory infections during the preceding six weeks and were stable at the time of study.

Patients with COPD complained of cough and sputum on most days in the month for years and exhibited moderate to severe (FEV_1/VC 20–60%) and irreversible airway obstruction (increase in FEV_1 < 10% after inhalation of 200 μg fenoterol) according to ATS criteria.[20] Before entering the study nine patients received daily inhaled corticosteroids and regular inhaled bronchodilator therapy. All bronchodilators were withheld for at least 12 hours before each study. No patients had had recent exacerbations defined as a change in quality and quantity of sputum with increased dyspnoea.

STUDY DESIGN

On day 1 a questionnaire on the characteristics and clinical scores of the patients was completed and baseline spirometric tests, skin prick tests to a battery of common aeroallergen extracts, and total serum IgE (RIST) were also performed. On day 2 the histamine inhalation test was performed. On day 3 sputum was induced in normal and asthmatic subjects and spontaneous sputum was collected from patients with COPD. Eighteen patients (10 asthmatics and eight with COPD) were asked to produce sputum on a second occasion within one week. All of the sputum from normal subjects and patients with asthma was collected as induced sputum, and all of the sputum from patients with COPD was spontaneously expectorated.

LUNG FUNCTION TESTS

Baseline pulmonary function testing was performed by measuring static and dynamic lung volumes with a water sealed spirometer (Pulmonet Godart) as previously reported.[21] The normal values for lung volumes are those proposed by the European Community for Coal and Steel.[22]

BRONCHIAL CHALLENGE

A histamine aerosol inhalation test was performed in each patient. Increasing concentrations of histamine acid phosphate in normal phosphate buffered saline (prepared by the University Hospital Pharmacy) were inhaled from a DeVilbiss 646 nebuliser (DeVilbiss Co, Somerset, Pennsylvania, USA) driven at an airflow rate of 6 l/min, mean (SD) output 0.31 (0.03) ml/min, using the tidal breathing method. With this method 4 ml of solution were placed in the nebuliser and inhalation continued during tidal breathing over two minutes. Histamine solution was stored at 4°C and nebulised at room temperature. Normal phosphate buffered saline was inhaled first, followed at five minute intervals by histamine in doubling concentrations from 0.031 to 8 mg/ml. The test was stopped at the concentration of histamine which caused a decrease in FEV_1 of > 20% from saline (provocative concentration). From the log dose-response curve the concentration of histamine required to produce a 20% fall in FEV_1 from saline ($PC_{20}FEV_1$) was noted. Details of the technique have previously been described.[23]

INDUCTION AND ANALYSIS OF SPUTUM

The induction of sputum was performed according to the method of Pin *et al.*[15] Ten minutes after fenoterol inhalation (200 μg) hypertonic saline was nebulised with an ultrasonic nebuliser (Fisoneb) and inhaled for five minute periods up to 20 minutes. The concentration of saline was increased at intervals of 10 minutes from 3% to 4%. FEV_1 was measured every five minutes during inhalation of hypertonic saline solution. The sputum induction procedure did not cause troublesome symptoms and the FEV_1 did not decrease by more than 20% in any subject. Every five minutes subjects were asked to try to cough sputum into a Petri dish.

Macroscopic characteristics of the sputum were recorded, and the quality of the sputum sample was assessed according to the method of Pin[15] by the number of lower respiratory tract plugs and the extent of salivary contamination in cell counts. Plugs free from salivary contamination were then suspended in DTT (dithiothreitol) solution (0.1%) and incubated for 30 minutes at 37°C. The cells were centrifuged at 1500*g* for 10 minutes and resuspended in saline. Three sputum slides were then prepared for cytological examination by cytocentrifugation. The cells were air dried and stained with May-Grunwald-Giemsa stain and differential counts were determined by counting 200 non-squamous cells on each sputum slide.

STATISTICAL ANALYSIS

The reproducibility of duplicate measurements of the cell profile was assessed by the coefficient of repeatability.[24] Regression analysis was performed by Spearman's rank correlation coefficient. The significance of the differences between groups was assessed by Kruskal-Wallis analysis of variance, the Mann-Whitney U test and the Student's *t* test when appropriate. Bonferroni's adjustment (0.05/n test) for multiple testing was used. The relation between categorical data was determined by the χ^2 test. A p value of < 0.05 was considered to be significant.

Results

Table 1 shows the anthropometric and clinical characteristics of the three groups. The groups significantly differed in age, patients with COPD being the oldest. As might be expected, patients with COPD smoked much more than asthmatics. $PC_{20}FEV_1$ was similar in asthmatic patients and those with COPD. The level of serum IgE (range 12–5069 U/ml in asthmatic subjects and 2–1939 U/ml in those with COPD) did not differ significantly among the patients. Skin prick tests were positive, with a wheal diameter of 3 mm or more, in 27 asthmatic subjects and in one with COPD. FEV_1 (% predicted) was lower in patients with COPD and asthmatic patients than in normal subjects, being lower in patients with COPD than in asthmatics.

The total number of cells in the sputum was similar in the three groups (table 2, fig 1). The differential count showed more eosinophils in

Table 1 Anthropometric and clinical data

Group	Sex (M/F)	Age (years)	Smoke (pack years)	Current smokers (n)	$PC_{20}FEV_1$ (mg/ml)	IgE (U/ml)†	Skin tests (pos/neg)	FEV_1/VC (%)	FEV_1 (% predicted)	Inhaled steroids (no of patients)
Asthma (A) (n=43)	28/15	36.9 (18–65)	11 (13)	7	*0.29 (0.008–7.8)	529 (1053)	27/16	75.4 (14)	93.2 (20)	22
COPD (n=18)	16/2	66.1 (51–80)	45 (24)	6	*0.20 (0.03–0.59)	305 (619)	1/17	43.8 (11)	48.2 (19)	9
Normal (N) (n=20)	12/8	38 (20–65)	0	0	> 16	< 85	0/20	82 (6)	108 (9)	0
p (N *v* A)		0.31						0.75	0.21	
p (N *v* COPD)		< 0.0002						< 0.0001	< 0.0001	
p (A *v* COPD)		< 0.0001	< 0.0001	0.10	0.07	0.20	< 0.001	< 0.0001	< 0.0001	0.90

Values are arithmetic means or * geometric means. Values in parentheses are standard deviations or ranges.
† Normal range < 85 U/ml.

Table 2 Mean (range) sputum cell counts

	Cell count				
Group	Total ($\times 10^5$/ml)	Eosinophils (%)	Neutrophils (%)	Macrophages (%)	Lymphocytes (%)
Asthma (A)	20.3 (14–30)	14 (0–70)	14.4 (0–48)	67.4 (16–98)	3.1 (0–37)
COPD	21.2 (16–32)	0.8 (0–3.9)	47.5 (0.7–79.5)	49.6 (15.6–96.7)	2.2 (0–7.9)
Normal (N)	19.6 (12–32)	0.6 (0–2)	8.8 (1.4–18)	89 (73–97)	1.5 (0.4–3.4)
Kruskal-Wallis analysis of variance					
H	0.35	16.3	19.2	17.5	0.04
p	0.93	< 0.001	< 0.001	< 0.001	0.98
Mann-Whitney test					
p (N *v* A)	0.83	< 0.05	0.21	< 0.05	0.98
p (N *v* COPD)	0.52	0.82	< 0.05	< 0.05	0.97
p (A *v* COPD)	0.92	< 0.05	< 0.05	< 0.05	0.95

Table 3 Relationships between eosinophils and neutrophils in the sputum and clinical data

	FEV_1 (%)	FEV_1/VC	$PC_{20}FEV_1$ (mg/ml)	Clinical score	Smoking history (pack years)
Asthma					
Eosinophils (%)	p < 0.035 (r_s = −0.45)	p < 0.029 (r_s = −0.46)	p < 0.04 (r_s = −0.32)	p < 0.037 (r_s = −0.32)	p = 0.12
Neutrophils (%)	p = 0.87	p = 0.71	p = 0.25	p = 0.46	p = 0.62
COPD					
Eosinophils (%)	p = 0.57	p = 0.90	p = 0.28	—	p = 0.76
Neutrophils (%)	p = 0.48	p = 0.33	p = 0.50	—	p = 0.30

asthmatic patients than in normal subjects and patients with COPD, and significantly more neutrophils in patients with COPD than in asthmatic patients and normal subjects. Nonetheless, in 12 asthmatic patients the differential count of neutrophils was higher than in normal subjects. The number of macrophages was lower in patients with COPD than in asthmatics, and no difference was seen in the lymphocyte count in the three groups. The repeatability coefficients[24] of the differential cell counts were 9.2 for eosinophils and 11.6 for neutrophils.

Table 3 shows the relationships between cellular profile, clinical, and functional data. In asthmatic patients the eosinophil count (%) was directly related to clinical scores and inversely related to the percentage predicted FEV_1, FEV_1/VC and $PC_{20}FEV_1$. In patients

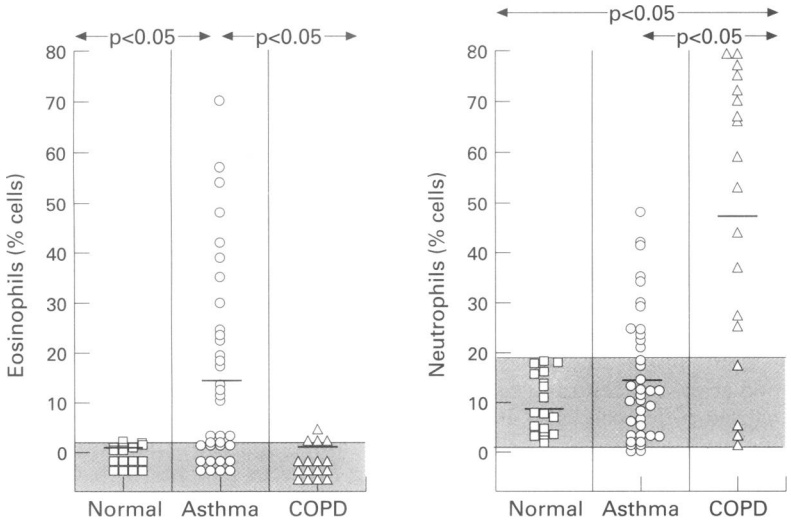

Figure 1 Differential cell counts of eosinophils and neutrophils in sputum of normal subjects and patients with asthma and COPD. Shaded area represents normal range. Horizontal bars represent mean values.

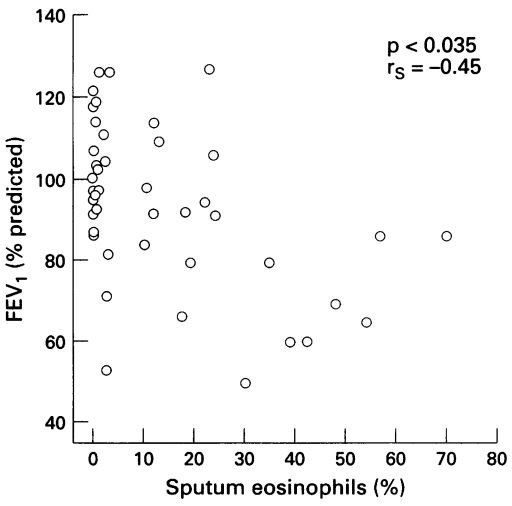

Figure 2 Relationship between eosinophils in sputum and baseline FEV_1 in patients with asthma.

Table 4 Clinical characteristics of asthmatic subjects according to the cytological profile

Subgroup	Subjects (n)	Age (years)	Smoking history (pack years)	IgE (U/ml)†	Skin tests (pos/neg)	$PC_{20}FEV_1$ (mg/ml)
AN	12	39.9 (16.7)	15.4 (20.3)	124 (139)	7/5	0.41*(0.008–6.3)
AWN	31	35.7 (13.6)	9.6 (8.5)	619 (1148)	20/11	0.24*(0.008–7.9)
p		0.49	0.96	0.1	0.1	0.23

AN = asthmatic subjects with increased sputum neutrophils; AWN = asthmatic subjects with normal range of sputum neutrophils. Values are arithmetic means and standard deviations of geometric means* and ranges.
† Normal range < 85 U/ml.

with COPD neither eosinophils nor neutrophils related to measures of airflow or $PC_{20}FEV_1$. The relationship between FEV_1 and the sputum eosinophil count is shown in fig 2.

Discussion

Our data show that chronic asthma was characterised by increased numbers of eosinophils in the sputum and COPD by increased numbers of neutrophils, although an increase in the numbers of neutrophils was also found in 28% of asthmatic patients. Sputum eosinophilia was related to FEV_1, FEV_1/VC and $PC_{20}FEV_1$ in asthmatic patients.

DATA ON EOSINOPHILS

A cellular inflammatory response in the bronchial mucosa characterised by eosinophil and mast cell infiltration plays an important part in the pathogenesis of asthma.[1–8 15 16 25–28] Our data are consistent with studies carried out using either bronchoalveolar lavage[1 2 4 27] or sputum.[15–17 25 28] We also found that increased levels of eosinophils in the sputum were related to baseline airway obstruction and severity of asthma as assessed in terms of both clinical scores and bronchial hyperresponsiveness, a pattern in line with previous reports with sputum,[15] bronchoalveolar lavage,[1 2 4 26] or biopsy specimens[6 29] from asthmatic patients without exacerbation, but in contrast to studies from patients during exacerbation of the disease.[30] In bronchial asthma inflammatory changes are thought to be central to bronchial hyperresponsiveness,[2 31] while in patients with COPD bronchial hyperresponsiveness is most likely to be the result of structural and geometric factors.[32 33] Our data, which show a significant but weak relationship between the eosinophil count and bronchial hyperresponsiveness in asthmatic patients but not in those with COPD, are in line with the above contention.

DATA ON NEUTROPHILS

Neutrophils have been reported to predominate in the sputum[34] and bronchoalveolar lavage fluid[10 26] of patients with COPD, but to be low both in the sputum of patients with stable non-obstructed chronic bronchitis[30] and in biopsy specimens from patients with COPD.[8 9 12] The difference between our data and those of others[30] is probably based on the selection criteria of the patients. In fact it has been shown that neutrophil levels in the airway are associated with airway obstruction.[10] According to Thompson *et al*[10] high levels of neutrophils were found exclusively in bronchoalveolar lavage fluid from patients with COPD, while in another study[26] low to intermediate levels of neutrophils were common in patients with non-obstructive chronic bronchitis and asthma. Based on previous observations,[13–15 34] a high number of neutrophils was not totally unexpected in asthma. In this regard, neutrophil inflammation during an asthmatic exacerbation has recently been reported to be more prominent than previously recognised.[13 14] On the other hand, an increased number of neutrophils has been reported in biopsy specimens from patients with sudden onset fatal asthma.[35] We were therefore careful to select patients in a stable condition and free of respiratory infections during the six weeks preceding the study. Despite this, we found an increased percentage of neutrophils in a subgroup of asthmatic subjects, a result in keeping with findings in bronchoalveolar fluid.[26] The clinical characteristics of asthmatic subjects with increased levels of sputum neutrophils did not differ from those of asthmatics with no increase in sputum neutrophils (table 4). Thus, the present data do not help in defining the contribution of neutrophils to asthma.

In conclusion, examination of sputum is a potential tool for clinical studies aimed at defining the cytological profile and evaluating interrelations between cellular and clinical patterns of airway inflammation in patients with asthma and COPD.

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