

Comparison of cellular and protein changes in bronchial lavage fluid of symptomatic and asymptomatic patients with red cedar asthma on follow-up examination

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Summary

Seventeen patients with occupational asthma due to western red cedar had bronchial lavage during follow-up examination after removal from exposure for at least 1 year. Seven patients were asymptomatic while ten continued to have symptoms of asthma requiring treatment. Symptomatic patients had evidence of airway inflammation, as reflected by a significantly higher total cell count, neutrophils and eosinophils, as well as an increase in protein and albumin in their bronchial lavage fluid compared to those without symptoms. Asymptomatic patients had no evidence of airway inflammation in the lavage fluid. There was no correlation between the degree of non-specific bronchial hyperresponsiveness and the number or percentage of inflammatory cells to suggest that cellular infiltration is the sole cause of persistent bronchial hyperresponsiveness.

Introduction

Several follow-up studies of patients with occupational asthma due to different agents have shown that more than half of the patients failed to recover after they left the industry and were no longer exposed to the offending agent [1–8] even after a period of many years. The persistence of asthmatic symptoms was accompanied by a heightened degree of bronchial responsiveness [2–5]. The cause of the persistent bronchial hyperresponsiveness in these patients is not known. Airway inflammation has been postulated as a cause for an increase in non-specific bronchial hyperresponsiveness [9]. There are now several studies, using the bronchoalveolar lavage (BAL) technique, that show an increase in the number of inflammatory cells in the BAL fluid during late

asthmatic reaction induced by allergens [10–12]. Late asthmatic reaction has been shown to be associated with an increase in non-specific bronchial hyperresponsiveness [13]. In addition, neutrophils are increased in BAL of subjects at a time when airway hyperresponsiveness was present after inhalation of ozone [14] and toluene diisocyanate [15].

In this report we present the data on bronchial lavage findings in patients with red cedar asthma who failed to recover after the removal from exposure, and compare them with those who recovered, in an attempt to find out whether persistent symptoms and bronchial hyperresponsiveness are related to the presence of persistent airway inflammation.

Subjects and methods

Subjects

Seventeen patients with red cedar asthma documented by inhalation provocation test in the past took part in the study. All of them had been away from exposure for over 1 year. Seven of them recovered with no symptoms and did not require medication. They became asymptomatic within a few weeks or months after removal from exposure. The remaining ten patients continued to have recurrent attacks of asthma after they were removed from exposure for more than a year and required medication for control of their symptoms. The medications required varied from patient to patient. One required the use of inhaled bronchodilator only intermittently; others required regular inhaled bronchodilator therapy with or without oral theophylline. Six patients required inhaled beclomethasone in addition to bronchodilators for the control of symptoms. The dose of beclomethasone used was 400 µg/day

Methods

All patients were carefully interviewed about their clinical symptoms, medication requirements and possibility of exposure to red cedar dust after they left the industry.

Spirometry was done on all patients using a 13.5 L Collins spirometry in a standardized manner [16]. Forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) were measured. The predicted values were obtained from Crapo *et al.* [17].

A methacholine inhalation test was carried out on the patients as described previously [18]. Aerosols of phosphate buffered saline followed by increasing concentrations of methacholine (0.025–32 mg/ml) were given to the patients by inhalation for 2 min, and FEV₁ was measured at 30 sec and 4 min after each inhalation; a positive response was indicated by a decrease in FEV₁ of more than 20% from the lowest post-saline level. The provocative concentration of methacholine that induced a 20% decrease in FEV₁ (PC₂₀) was determined from the dose–response curve. The methacholine inhalation test conducted in our laboratory is highly reproducible [18].

Bronchial lavage was carried out as described previously [19]. Briefly, fiberoptic bronchoscopic examination was performed following premedication with 0.8 mg atropine i.m. and local anaesthesia with 2% lidocaine (Xylocaine). Sterile isotonic saline solution warmed to body temperature was used for lavage. The tip of the bronchoscope was wedged into a segmental bronchus in the right middle or lower lobe. Aliquot (20 ml) was instilled into a subsegmental bronchus as a single bolus, the fluid was recovered by a wall-mounted suction apparatus using 100 mmHg negative

pressure. The volume of fluid recovered was measured. A total cell count was made with a haemocytometer. The fluid was centrifuged at $500 \times g$ for 15 min. The cell-free supernatants were decanted for measurement of proteins. The cell pellet was resuspended. A cytocentrifuged preparation was made and the cells were stained with haematoxylin and eosins for a differential count. The proportions of alveolar macrophages, lymphocytes, neutrophils, eosinophils, bronchial epithelial cells and degenerated cells were calculated. A minimum of two slides were made from each aliquot. The slide with the most even distribution of cells was used for the differential count. The cells were counted from left to right and from top to bottom. A total of 500 cells were counted.

Total protein was quantitated with the BioRad method (Bio Rad Laboratories, Rockville Center, NY, USA) in duplicates. The results of the duplicates were within 10% of each other. Albumin was quantitated using low level immunodiffusion plates (Behringwerke AG, FRG). The lower limit for the assays was 100 $\mu\text{g/ml}$ for total protein and 5 $\mu\text{g/ml}$ for albumin. All proteins were quantitated in unconcentrated fluids [19].

Standard statistical methods were used. For comparison of data between symptomatic and asymptomatic subjects, Student's unpaired *t*-test was used. For correlation of PC_{20} and various cell counts, linear regression analysis was used after logarithmic transformation of PC_{20} .

Results

The clinical features of the two groups of patients are shown in Table 1. They were all life-long non-smoking males. The patients who became asymptomatic were younger and diagnosed earlier, compared with the patients who remained symptomatic. The asymptomatic patients, as expected, had significantly ($P < 0.01$) better lung function and less degree of bronchial hyperresponsiveness compared with the symptomatic patients at the time of the follow-up examination. Two asymptomatic and two symptomatic patients were atopic (positive immediate skin reaction to one or more common allergens).

The percentages of lavage fluid recovered among the asymptomatic and symptomatic groups were $47 \pm 28\%$ and $40 \pm 20\%$, respectively. They were not statistically different. The cellular and protein contents of the bronchial lavage fluid of the patients are shown in Table 2. The total cell count was significantly higher among the symptomatic patients. The percentages of neutrophils, eosinophils, epithelial and degenerated cells were also higher in this group of patients; the differences between the two groups reached the level of statistical significance for neutrophils, eosinophils and degenerated cells. The total protein and albumin concentrations were also significantly higher among symptomatic patients compared with the asymptomatic patients. There was no correlation between PC_{20} and the absolute cell counts, the differential cell counts, total protein or albumin concentration of the lavage fluid. There was no significant difference in the PC_{20} or the total and differential cell counts between patients who were taking inhaled steroids and those who were taking bronchodilators alone.

Discussion

In this study we found that patients with red cedar asthma who failed to recover after the cessation of exposure had evidence of persistent airway inflammation, as reflected

Table 1. Characteristics of patients studied

Subject no.	Age (years)	Duration of symptoms before diagnosis	Duration away from exposure (years)	Atopic status†	FEV ₁ (%)		PC ₂₀ (mg/ml)		Medications§
					Initial	Follow-up	Initial	Follow-up	
<i>Asymptomatic</i>									
1	56	1	2	—	107	117	2.0	8.0	—
2	37	2	9	—	89	88	4.75	32.0	—
3	31	1.5	2	—	70	85	0.45	1.67	—
4	46	3	9	—	113	116	0.50	1.5	—
5	29	0.5	1	—	98	102	0.65	4.0	—
6	34	1	8	+	87	108	n.d.‡	16.0	—
7	37	1	9	+	89	105	n.d.	32.0	—
	38.6±9.4	1.4±0.8	5.7±3.8		93.3±14.2	103±12	1.67±1.23	13.6±13.5	
<i>Symptomatic</i>									
1	67	9	2	—	70	76	n.d.	n.d.	B, S, T
2	37	2	10	—	97	85	2.10	0.65	B, S
3	67	3	6	—	100	87	1.20	1.60	B, S
4	33	0.75	2	—	105	108	1.05	0.2	S
5	59	4	1	+	82	82	0.55	0.5	S
6	27	2	1	+	78	80	0.22	0.25	S, T
7	53	5	1	—	80	68	16.00	2.14	B, S, T
8	25	2	2	—	99	55	3.44	0.01	B, S
9	28	3	1	—	81	77	n.d.	1.00	B, S, T
10	37	2	2	—	100	85	1.21	0.50	S
	43.3±16.6	3.3±2.3*	2.7±3.0		89.2±12.2	80±14*	3.2±5.3	0.76±0.70*	

* Differences between symptomatic and asymptomatic patients is statistically significant by Student's unpaired *t*-test, *P* < 0.05.

† Atopic status + or — = positive or negative immediate skin reaction to one or more of the common allergens.

‡ n.d. = Not done.

§ B = Beclomethasone, S = Salbutamol, T = Theophylline.

Table 2. Cellular and protein content of bronchial lavage fluid of patients with red cedar asthma

Total cell count ($\times 10^4$)	Differential cell count (%)					Total protein ($\mu\text{g/ml}$)	Albumin ($\mu\text{g/ml}$)
	Macrophage	Lymphocyte	Neutrophils	Eosinophils	Epithelial cells		
<i>Asymptomatic</i>							
1 2.5	60	3	5	0	22	10	14
2 14.0	52	25	7	1	13	2	57
3 1.5	79	2	1	0.4	17	1	9
4 5.5	43	1	6	0	48	2	14
5 8.0	65	13	5	1	8	8	27
6 1.95	30.4	7.2	6.6	2.8	48.2	4.2	74
7 1.12	31.6	3.0	3.0	0.4	54.4	7.6	87
4.9 $\pm$ 4.7	51.6 $\pm$ 17.9	7.7 $\pm$ 8.6	4.8 $\pm$ 2.1	0.8 $\pm$ 0.9	30.0 $\pm$ 19.4	5.1 $\pm$ 3.5	40 $\pm$ 32
<i>Symptomatic</i>							
1 11.3	18	5	29	3	37	8	50
2 12.5	23	2	10	1.4	56	6	116
3 13.3	38	9	5	2	30	17	64
4 16.0	54	5	9	3.2	16	12	10
5 13.3	24	1.6	16.6	0	25	15	242
6 10.0	53	5	0.4	5.2	30	10	16
7 14.2	8	2.0	56.4	6.8	23.2	3.6	173
8 9.45	52	9.4	3.2	1.2	18.8	15.4	127
9 12.5	80	10	0.8	2.8	1.4	4.6	94
10 9.0	17.0	3.4	50.0	15.2	22.6	1.6	77
12.1 $\pm$ 2.2*	36.7 $\pm$ 22.6	5.2 $\pm$ 3.2	18.0 $\pm$ 20.4*	4.8 $\pm$ 4.4*	26.0 $\pm$ 14.3	9.3 $\pm$ 5.4*	97 $\pm$ 71*
						313 $\pm$ 287*	

* Differences between symptomatic and asymptomatic is statistically significant by Student's unpaired *t*-test, $P < 0.05$.

by a higher total cell count, neutrophils, eosinophils and increased protein and albumin content in the lavage fluid. The cellular and protein contents of the bronchial lavage fluid of these patients were not different from the group of patients with intrinsic asthma [20]. The findings in the bronchial lavage fluid of the asymptomatic patients were not any different from those of the healthy non-smoking controls in our previous studies [20] even though four of the eight asymptomatic patients had a mild to moderate degree of bronchial hyperresponsiveness. In the symptomatic patients the number and percentage of neutrophils and eosinophils were elevated. Our findings were essentially similar to a preliminary study of patients with toluene diisocyanate-induced asthma who failed to recover after removal from exposure [21].

We were unable to find a significant correlation between the degree of non-specific bronchial hyperresponsiveness and the total or differential cell counts to suggest an association between cellular infiltration and persistent bronchial hyperresponsiveness. This is in contrast to the findings by Kirby and co-workers [22] who observed a significant correlation between the total cell count, the number of metachromatic cells or eosinophils in the lavage fluid and the level of bronchial hyperresponsiveness in subjects with mild stable asthma not receiving regular treatment. This lack of correlation in our study is unlikely to be due to the fact that six patients were receiving inhaled steroids. Ryan *et al.* [23] demonstrated only a small increase in PC₂₀ in a group of patients with asthma after inhaled steroids. Moreover, we were unable to find any difference in the PC₂₀ and cell counts between patients who were taking inhaled steroids and those who were taking bronchodilators alone. The lack of association between cellular infiltration and bronchial hyperresponsiveness should not be surprising because cellular infiltration is only one of several causes of increase in bronchial wall thickness, which in turn can influence bronchial hyperresponsiveness [24]. Bronchial oedema and an increase in airway blood volume cannot be assessed by bronchial lavage. In addition, there are a number of other factors that can alter the degree of bronchial hyperresponsiveness such as airway smooth muscle hypertrophy, an increase in the proportion of muscle in the bronchial wall circumference, an increase in mucus production or mucus plugging, and an alteration in the size of the cartilage plates or the mechanical properties of the airway cartilage [24]. Furthermore, cell numbers may not reflect cell function although the study by Kirby and co-workers [22] did not find an association between bronchial responsiveness and eosinophil granule-derived products. It may be that the state of activation of the cells is more important than the absolute number of cells. The finding in the present study that four of the seven asymptomatic subjects had mild to moderate degree of bronchial hyperresponsiveness to methacholine on follow-up, without an increase in protein or inflammatory cells in their lavage fluid, suggests that factors other than airway inflammation may play an important role in the persistence of their bronchial hyperresponsiveness.

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