

TABLE I. Subjects' characteristics

Subject	Age (yr)	Sex	FEV ₁		FVC		Atopic status*
			L	(% pred)	L	(% pred)	
1	29	M	4.72	(114.3)	5.53	(109.3)	T, R
2	48	F	3.22	(120.6)	3.78	(109.9)	G, R, C, H, HD
3	33	M	4.59	(105.3)	5.40	(98.4)	T, G, C
4	21	F	3.06	(95.9)	3.37	(92.6)	G, R, C, C, D, H, HD
5	30	M	4.44	(101.1)	5.81	(106.6)	T, G, C, D
6	22	M	4.85	(110.7)	5.16	(99)	T, G, R, C, HD
7	31	F	2.68	(92.4)	2.76	(80.9)	R, HD, C
8	32	M	4.53	(107.3)	5.47	(101.3)	G, HD, C, D, Ho
9	31	F	3.18	(109.7)	3.43	(100.6)	T, G, R, C
10	23	F	3.39	(106.3)	4.00	(108.7)	T, D, HD

*Positive (>2 mm) wheal-and-flare response to prick tests with common airborne allergens.

T, trees; G, grasses; R, ragweed; HD, house dust; C, cat; D, dog; H, horse; Ho, *Hormodendrum*.

"borderline" airway responsiveness.^{11, 12} They appear more susceptible to develop asthma than normal subjects, possibly in relation to repeated antigenic exposure.^{12, 13} Previous studies have demonstrated that airway responsiveness may increase in subjects without asthma with allergic rhinitis after laboratory or natural exposure to antigens to which they are sensitized.^{14, 15} Allergic rhinitis may, in some subjects, be a predisposing condition to the development of asthma.^{11, 13, 16}

The mechanisms by which atopic subjects with initially normal NAAR develop asthma remain undefined. Repeated antigenic exposure could probably induce inflammation of the airways and eventually hyperresponsiveness. Studies on the effects of chronic exposure to dust mite on airway responsiveness support this hypothesis.¹⁷ These authors have demonstrated a reduction in airway hyperresponsiveness after prolonged allergen avoidance.

In this study, we searched for evidence of lower airway inflammation in BAL of subjects without asthma with pollen-induced allergic rhinitis during seasonal exposure to antigens to which they were sensitized. The purpose of the study was to observe if changes on BAL cell-differential analysis correlated with changes of airway responsiveness to MC.

METHODS

Subjects

We studied 10 adults (five male and five female subjects) without past or present evidence of asthma. Nine adults were nonsmokers, and one subject (No. 7, Table I) smoked 10 to 15 cigarettes a day. He stopped smoking for the duration of the study. All subjects had a history of recurrent

summertime rhinoconjunctivitis. All subjects had normal expiratory flows (Table I). Mean FEV₁ ± SEM was 3.87 ± 0.26 L (106.4% ± 2.7% predicted) and mean FVC, 4.47 ± 0.35 L (100.7% ± 2.8% predicted). Skin prick tests with 26 common airborne allergens demonstrated wheal-and-flare responses to tree, grass, and/or ragweed pollens (Table I). Out of pollen season, these subjects were asymptomatic and had no exposure to sensitizing agents other than house dust. The level of sensitization to house dust mites, as assessed by the mean wheal diameter on skin prick tests, was quite less (3.6 ± 0.4 mm) than for pollens (9.2 ± 1.4 mm). The only medication administered during the study was nasal flunisolide, astemizole, or terfenadine, which were taken at the peak of symptoms by subjects Nos. 5, 9, and 6, respectively, for periods varying from 3 to 8 days. The study was approved by the Laval Hospital Ethics Committee, and all subjects gave written informed consent.

Study design

The subjects were studied between March and November 1988. Symptoms of rhinoconjunctivitis and cough in and out of the pollen season were recorded daily, on a scale 0 to 5: 0, no symptom; 1, very light; 2, light; 3, moderate; 4, severe; and 5, very severe.¹⁸ Mean daily scores for symptoms of rhinitis (sneezing, nasal obstruction, pruritus, and rhinorrhea) and conjunctivitis (ocular pruritus, swelling, pricking, and redness of the eyes) were totaled, and the mean was determined to obtain an average daily score for symptoms of rhinoconjunctivitis. PEFs were measured daily in the morning and at bedtime. The best of three consecutive recordings was noted on a diary card.

The subjects reported to the laboratory each month for an MC inhalation test. A flexible bronchoscopy and BAL was done at the peak of nasal symptoms, before the administration of any medication, and either before or after the pollen season. At the time of bronchoscopy, blood was drawn for the determination of eosinophil counts.

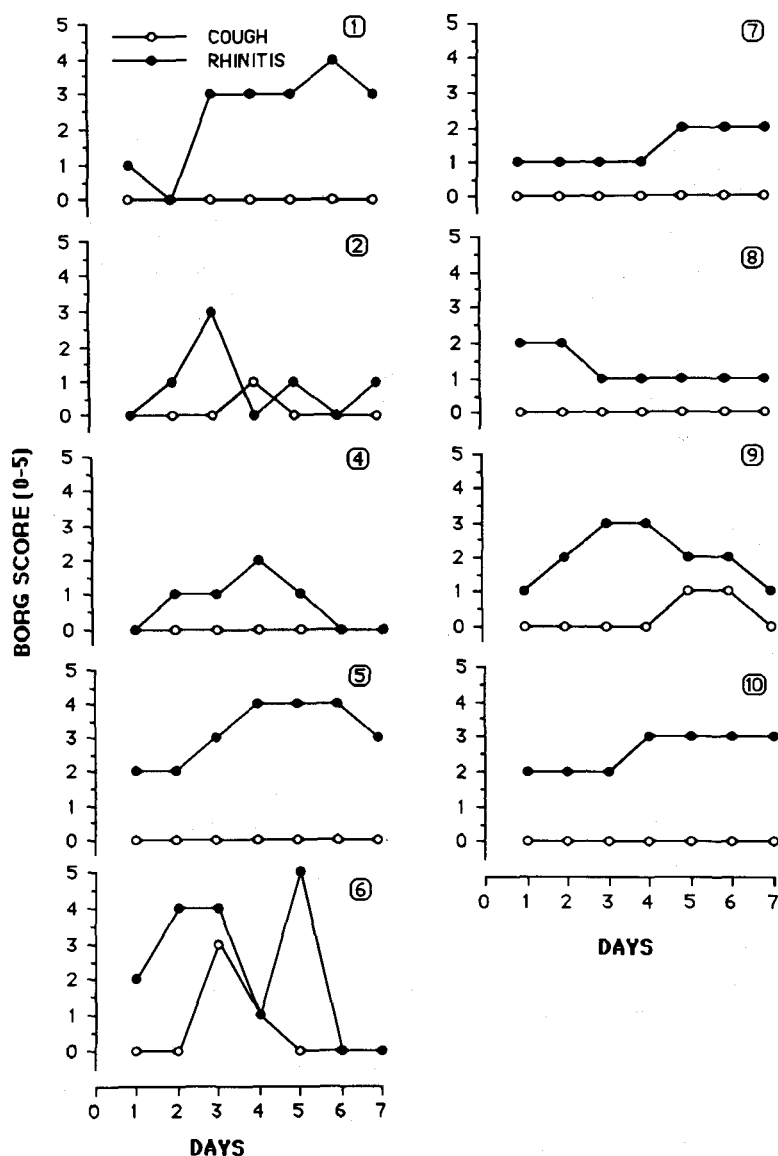


FIG. 1. Symptoms of rhinoconjunctivitis and cough in pollen season during the week before BAL. All subjects developed symptoms of rhinoconjunctivitis during natural exposure to pollen. Four subjects reported a cough during this period. Subject No. 3 had symptoms of rhinoconjunctivitis at the time of the lavage, but symptoms were not scored.

MC inhalation tests

Nonallergic bronchial challenges were performed according to the method of Cockcroft et al.¹⁹ MC was prepared at doubling concentrations from 8 to 256 mg/ml and administered for 2 minutes at 5-minute intervals via a face mask, at an output of 0.13 ml/min. The responses were expressed as the PC_{20} , obtained by interpolation from the log dose-response curve.

BAL and cell differential

BAL was done under local anesthesia with a flexible bronchoscope.²⁰ The bronchoscope was wedged in a segmental or subsegmental bronchus of the right middle lobe,

and three 50 ml aliquots of normal saline (37° C) were instilled and aspirated with a syringe via the bronchoscope channel. The fluid was filtered through a gauze to remove mucus and kept on ice until it was processed. The lavage fluid was centrifuged (4° C for 10 minutes at 500 g), and the cell pellet was resuspended in Hanks' balanced salt solution or in phosphate-buffered saline.

The total number of cells was estimated on hemocytometer. Cell cytocentrifuge preparations were stained with Diff-Quik (American Scientific Products, McGraw Park, Ill.) and nonspecific esterase stains. Cell differentials were obtained by counting 300 cells on each preparation. Search for ciliated epithelial cells was done by counting 1000 cells

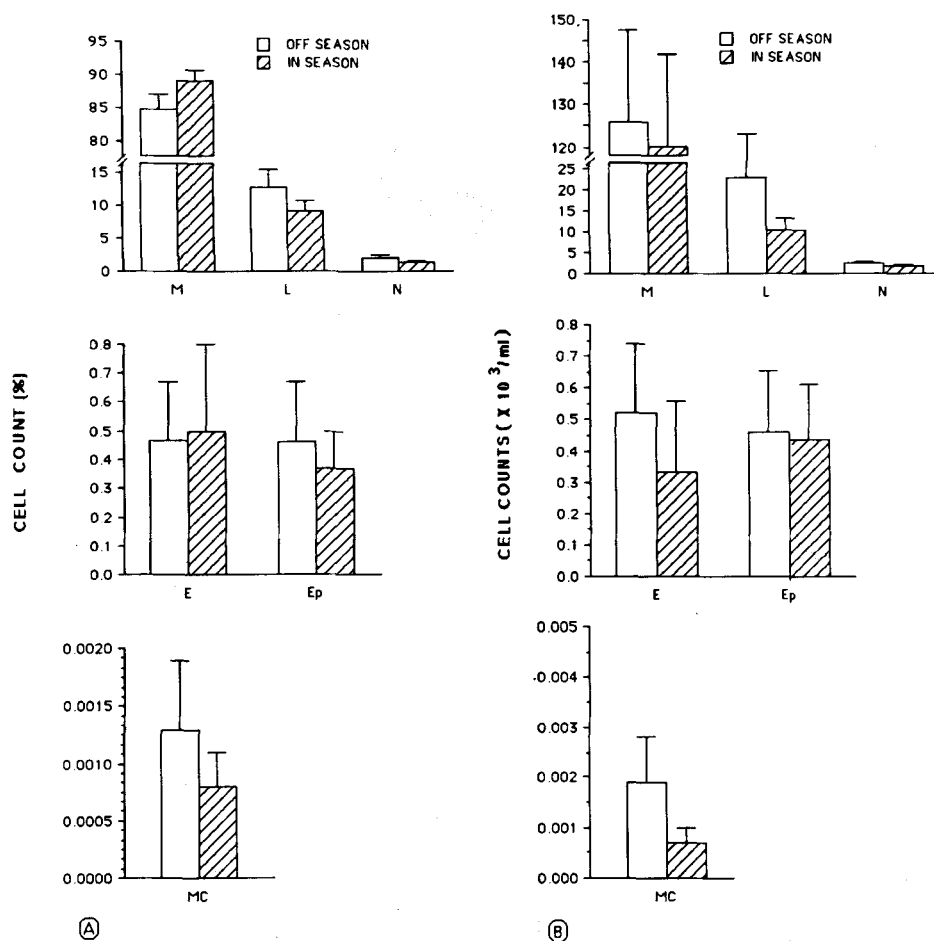


FIG. 2. BAL cell-differential counts expressed. **A**, In percent. **B**, In cells per milliliter in and out of pollen season. Macrophage (*M*), lymphocyte (*L*), neutrophil (*N*), eosinophil (*E*), epithelial cell (*EP*), and mast cell (*MC*) counts did not change significantly during antigenic exposure, suggesting that there was no underlying airway inflammation.

on Diff-Quik-stained preparations. Metachromatic cell count was done with toluidine blue-stained preparations and counting at least 1000 cells. Since most of the lavage metachromatic cells are known to be mast cells, they were reported as such.

Statistical analysis

Values are expressed as mean $\pm$ SEM. Nonparametric tests (Wilcoxon rank test) were used to compare BAL cell-differential counts, blood eosinophil counts, and the lowest PC₂₀ MC during the pollen season and out of season. A significant change in PEF was defined as a $>20\%$ reduction from baseline. To allow comparisons of data in all subjects, a PC₂₀ value >256 mg/ml was considered as equal to 256, although this could underestimate the observed changes.

RESULTS

The mean daily symptom scores of each subject (except No. 3) for cough and rhinoconjunctivitis during the week preceding the BAL in season are ex-

pressed in Fig. 1. All subjects developed seasonal symptoms of rhinoconjunctivitis of variable intensity. The score for rhinoconjunctivitis went from 0 initially to between 1 and 5 at the peak of allergenic exposure (maximum score: light, two subjects; moderate, three subjects; severe, two subjects; very severe, one subject). Four subjects developed a cough during this period. Subject No. 3 experienced symptoms of rhinoconjunctivitis in the days before and at the time of the lavage but did not score the symptoms. At the in-season day of BAL, eight subjects had concurrent symptoms of allergic rhinitis and two subjects had symptoms 2 days before. No dyspnea, chest tightness, or wheezing were reported.

Out of season, the volume of BAL fluid recovered was $67.3\% \pm 2.1\%$ of the infused volume, compared to $59.9\% \pm 2.0\%$ during antigen exposure ($p > 0.05$). The mean total number of recovered cells with BAL was $23.2 \pm 3.5 \times 10^6$ ($13.3 \pm$

TABLE II. Seasonal changes in flow rates and airway responsiveness in and out of the pollen season

	PC ₂₀ (mg/ml)‡								Eosinophils			
	PEF* (L/min)		FEV ₁ (L)†		Nearest of BAL		Lowest		Blood ($\times 10^6$ /L)		BAL (%)	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Subject												
1	565.3	573.1	4.72	4.74	42	67	34	66	272	64	1	0
2	441.8	450.3	3.19	3.10	186	>256	186	>256	35	53	0	0
3	NM	NM	4.56	4.50	114	152	70	152	188	108	1	1
4	446.4	448.4	3.21	3.12	28	56	28	32	105	35	3	2
5	638.0	654.6	4.22	4.24	>256	>256	>256	>256	70	65	0	0
6	621.0	627.4	4.83	4.97	>256	>256	>256	>256	0	70	0	0
7	417.1	422.5	2.39	2.43	115	171	94	164	123	96	0	0.7
8	601.0	596.7	4.53	4.43	>256	>256	>256	>256	194	158	0	0
9	471.1	441.9	3.20	2.89	53	111	44	77	211	123	0	0
10	399.3	387.6	3.39	3.30	36	22	36	22	157	256	0	1
Mean	511.2	511.4	3.82	3.77	81.2	105.9	88.8	114.1	135.5	102.8	0.5	0.5
± SEM	31.4	33.5	0.27	0.28	2.4	2.4	2.5	2.5	26.8	20.6	0.3	0.2

NM, Not measured.

*Mean of PEF measured twice a day during 118.6 ± 15.6 days in season and 85.1 ± 11.6 days out of season.†Baseline FEV₁ value measured at the monthly visit.

‡Mean of two to four measurements.

2.3×10^4 cells per milliliter) in season and $33.8 \pm 7.4 \times 10^6$ ($15.2 \pm 3.1 \times 10^4$ cells per milliliter) out of season ($p > 0.05$). BAL cell differentials (percent) for macrophages, lymphocytes, neutrophils, eosinophils, and metachromatic cells in percent were, respectively, 89.0, 9.1, 1.3, 0.5, and 0.0008 during exposure to pollen and 84.6, 12.8, 2.1, 0.5, and 0.0013 out of season (Fig. 2). These differences were not significant. The mean number of epithelial cells recovered on BAL was not different with, respectively, $0.37\% \pm 0.13\%$ and $0.46\% \pm 0.21\%$ (0.43 ± 0.18 and $0.46 \pm 0.19 \times 10^3$ cells per milliliter) in and out of season.

Although the eosinophil count increased in seven subjects in season, the mean number of blood eosinophils for the whole group did not increase significantly during antigenic exposure compared to the out of season period. Blood eosinophil counts taken in and out of season were, respectively, $135.5 \pm 26.8 \times 10^6$ /L and $102.8 \pm 20.6 \times 10^6$ /L ($p > 0.05$).

PEF and pre-MC FEV₁ were similar in and out of the pollen season. Baseline FEV₁ out of season was 3.77 ± 0.28 L compared to 3.82 ± 0.27 L, the lowest measured during antigen exposure (Table II).

Overall airway responsiveness to MC increased during antigenic exposure compared to out of season, although differences in the mean PC₂₀ in and out of season for the group of 10 subjects did not reach

statistical significance. Mean PC₂₀ was 98.8 in season compared to 121.4 out of season ($p > 0.05$). In each case, MC responsiveness remained in the normal range throughout the study.

Four subjects increased their NAAR to MC in season, decreasing their PC₂₀ by at least one double concentration. This subgroup of subjects had a 1.7-fold increase in blood eosinophils during antigenic exposure, although the absolute cell count could still be considered in the normal range. Their BAL eosinophil count was unchanged during the pollen season.

DISCUSSION

The current study observed the relationship between the increase in MC responsiveness and lower airway inflammation in subjects with allergic rhinitis without asthma. No evidence of lower airway inflammation was found in BAL fluids during natural exposure to pollen in sensitized subjects who developed significant symptoms of allergic rhinoconjunctivitis. There was a small increase in airway responsiveness to MC, although this was not statistically significant for the whole group. It is difficult to assess the changes in PC₂₀ in such a group of subjects since many (four) had a PC₂₀ >256 mg/ml. This does not exclude that these last subjects may have changed airway responsiveness at a level of PC₂₀ >256 mg/ml. However, some subjects had more than one doubling concentration decrease in PC₂₀ during antigen exposure and

did not demonstrate any evidence of airway inflammation.

These results support previous observations demonstrating that NAAR may increase during antigenic exposure in atopic subjects without asthma with normoresponsive airways.^{14, 15} In a previous study on the effects of antigenic exposure in subjects with allergic rhinitis, we have demonstrated that usually this increase in airway responsiveness is mild, remains in the normal range, and is not associated with classic symptoms of asthma. In this previous study in which no evaluation of airway inflammation was done, we observed significant changes in PC₂₀ during the pollen season in five of eight subjects. The present observations were done with different subjects and during another season. The magnitude of changes in PC₂₀ are slightly less in this second study. We believe, however, that this finding reflects the variability of the airway response to natural exposure to antigens, influenced by many factors, such as the level of pollen exposure and interindividual differences.

IgE-mediated reactions in subjects with rhinitis may occur not only in upper airways but also in the lower airways. In the lower airways they may be subclinical and insufficient to induce airway obstruction and asthma symptoms. The lack of changes in cell-differential counts on BAL suggests, however, that natural antigenic exposure is not sufficient to induce airway inflammation in atopic subjects without asthma. The changes in airway responsiveness observed are therefore probably not due to airway inflammation. It is possible that inflammation of airway mucosa was present without any significant changes in cells present in the airway lumen. Bronchial biopsy specimens could have revealed inflammatory changes in the bronchial wall that may remain undetected by BAL. However, BAL has been a very useful tool to obtain representative samples of alveolar cell population from the airways and to document inflammatory reactions after acute antigen exposure in sensitized subjects with asthma.²¹⁻²⁶

Our mostly negative findings may be related to the intensity of the stimulus. It is difficult to quantify the degree of antigenic exposure in our subjects. There could be a threshold of exposure under which there is no detectable change in airway cells. However, all subjects developed significant symptoms of rhinoconjunctivitis, suggesting significant exposure. They had a symptom score ranging from 1 to 5 during the pollen season. Two subjects had no symptom at the time of the BAL, but they had significant symptoms on the previous days.

BAL provides a punctual measurement of lung cells, and if airway inflammation is transient, it could

have been missed at the time of the lavage. However, in most subjects, the lavage was performed at the peak of allergic symptoms, during antigen exposure, usually at the time NAAR was increased. The airway eosinophilic infiltrate after antigen exposure lasts from a few hours to 3 to 10 days in animal models, and it is probable that it follows the same pattern in man.^{27, 28} Then, even if the allergen-induced airway inflammation was short lasting, it should have been detected in our study.

Furthermore, mediators such as leukotrienes or platelet-activating factor, have been involved in changes in NAAR.^{29, 30} We do not know if the release of such mediators in the peripheral blood, after an upper airway allergic reaction, could influence airway responsiveness. Nonallergic mechanisms may also be involved. The upper airways could contribute to the increase in responsiveness through a reflex pathway. The presence of nasobronchial or orobronchial reflex has been suggested.^{31, 32} Impaired humidification and warming of inhaled air in the upper airways due to nasal occlusion and/or reduction in the filter effect of the nose with mouth breathing could result in exposing the airways to higher concentrations of antigenic particles.³²

Although some of our subjects changed their NAAR, they did not have changes in expiratory flows nor did they develop asthma symptoms during the study. This may be explained by the fact that our subjects had normal baseline airway responsiveness and that this increase in responsiveness did not reach the asthmatic range. If the intensity and/or the duration of exposure was higher, a level of NAAR at which a subsequent antigenic stimulus could induce changes in FEV₁ ("priming effect") might be reached. This could happen when NAAR is close to the asthmatic range. Mild increases in responsiveness can then heighten airway responsiveness to a level at which asthma symptoms are usually observed. In conclusion, our study demonstrates that antigenic exposure leading to symptoms of allergic rhinitis does not induce airway inflammation detectable on BAL.

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