

Eosinophilic airway inflammation in nasal polyposis

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Background: Asthma and asymptomatic bronchial hyperresponsiveness (BHR) are frequent findings in patients with nasal polyposis (NP).

Objective: To elucidate mechanisms responsible for the development of BHR, we initiated a prospective study of bronchial inflammation as assessed by bronchial lavage (BL) and bronchial biopsy specimens in 35 patients with noninfectious NP.

Methods: BHR was determined with methacholine provocation testing. Differential cell count, ECP, and histamine and tryptase levels were determined in BLs. Pathologic examination of bronchial biopsy specimens was performed with May-Grünwald-Giemsa stain to assess the number of lymphocytes. Indirect immunoenzymatic methods were used to identify eosinophils and mast cells.

Results: Fourteen patients did not exhibit BHR (group A); 7 patients had asymptomatic BHR (group B); and 14 patients had BHR associated with asthma (group C). Patients of group C tended to have a longer duration of nasal symptoms than those of groups A and B. FEV₁ (L) was significantly lower in group C than in groups A and B. The number and percentage of eosinophils were significantly higher in BLs in groups B and C than in group A ($P < .05$). Patients of groups B and C had a significantly higher number of eosinophils in bronchial submucosa ($14.0 \pm 1.5/\text{mm}^2$ and $19.0 \pm 1.9/\text{mm}^2$, respectively) than patients of group A ($0.1 \pm 0.1/\text{mm}^2$). The number of lymphocytes was also higher in groups B and C than in group A. FEV₁ (percent of predicted value) and eosinophil number within bronchial mucosa correlated negatively.

Conclusion: Our results demonstrate that patients with NP and asymptomatic BHR had an eosinophilic bronchial inflammation similar to that observed in asthmatic patients with NP, whereas patients with NP without BHR do not feature eosinophilic lower airways inflammation. The clinical relevance of these results requires careful follow-up to determine whether eosinophilic inflammation in these patients precedes and is responsible for the development of obvious asthma. (*J Allergy Clin Immunol* 1999;104:85-92.)

Key words: Nasal polyposis, bronchial hyperresponsiveness, eosinophil, bronchial inflammation, bronchial lavage, bronchial biopsy

Abbreviations used

BHR:	Bronchial hyperresponsiveness
BL:	Bronchial lavage
ECP:	Eosinophil cationic protein
FEF ₂₅₋₇₅ :	Maximal midexpiratory flow
FVC:	Forced vital capacity
NARES:	Nonallergic rhinitis with eosinophilia syndrome
NP:	Nasal polyposis
UAD:	Upper airways disorders

Nasal polyposis (NP) is a chronic inflammatory disease of the paranasal sinus mucosa, leading to the protrusion of edematous polyps into the nasal cavities responsible for persistent nasal obstruction. Histologically, nasal polyps are characterized by an edematous stroma filled with a prominent eosinophilic infiltrate underneath a thickened basement membrane.^{1,2} They can be primary or take part in different diseases.³ Lower respiratory tract disorders, such as asthma and nonspecific bronchial hyperresponsiveness (BHR), are associated in high frequency with NP.⁴⁻⁶ Recent reports have shown that approximately 30% of patients with NP had asthma. Several authors⁷⁻⁹ have also reported that patients with NP and no clinical evidence of asthma commonly exhibited nonspecific BHR. In a recent prospective study of patients with noninfectious NP, we reported that 17 of 22 patients with NP and nonspecific BHR had asthma, whereas 5 patients had asymptomatic BHR.¹⁰ The significance of asymptomatic BHR associated with chronic inflammatory nasal diseases is unclear. It has been suggested that nonspecific BHR may represent an indication of potential asthma.

It is well established that the most characteristic feature of asthma is an eosinophilic bronchial inflammation leading to nonspecific BHR.¹¹⁻¹³ Several studies clearly reported an increased number of eosinophils and lymphocytes in the bronchial mucosa of subjects with allergic and nonallergic asthma. By contrast, data concerning the relationship between asymptomatic BHR and the presence of inflammatory changes in the lower airways of nonasthmatic patients are controversial.¹⁴ In healthy subjects with no previous history of asthma or rhinitis, asymptomatic BHR does not seem to be associated with significant inflammatory bronchial changes.^{15,16} On the other hand, recent studies demonstrated that asymptomatic BHR was associated with an increased number of

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Supported by Programme Hospitalier de Recherche Clinique (PHRC, 1997, No 1916) du CH et U de Lille.

Received for publication Oct 26, 1998; revised March 2, 1999; accepted for publication March 2, 1999.

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0091-6749/99 \$8.00 + 0 1/1/98207

eosinophils in induced sputum from subjects with allergic rhinitis¹⁷ or with nonallergic rhinitis with eosinophilia syndrome (NARES).¹⁸

Little is known about the pathogenesis of asthma and asymptomatic BHR associated with NP. The possibility that a similar bronchial inflammation process could be involved in the BHR that is associated with NP has not been yet investigated. Our hypothesis was that asymptomatic BHR in patients with NP was related to a subclinical inflammation similar to that which we observed in asthmatic patients with NP. To establish this, we initiated a prospective study of bronchial inflammation in bronchial biopsy specimens and bronchial lavages in 35 consecutive patients with noninfectious NP. We also looked at a possible correlation between clinical findings of NP and airway inflammation.

METHODS

Patients

We studied 35 patients (20 male and 15 female patients) consecutively referred to the Calmette Hospital for pulmonary evaluation of noninfectious NP. The mean $\pm$ SEM age was 40.3 ± 1.9 years. Nasal polyps were identified in all patients by the following criteria: nasal symptoms (obstruction, anosmia, sneezing, rhinorrhea, itching) and visualization of polyps by anterior rhinoscopy. Each nasal symptom was scored from 0 to 3 (0 = no symptom; 1 = mild symptom [just noticeable]; 2 = moderate symptom [annoying]; 3 = severe symptom [distress]) so that maximal nasal score was 15 of 15. All patients were treated with topical steroids (beclomethasone, 2 to 4 puffs in each nostril twice a day). Patients with infectious sinusitis and/or previous history of nasal surgery were excluded from the study.

Study protocol

The study protocol was approved by a local ethics committee, and informed consent was obtained from all the patients. Subjects attended the laboratory on 2 different occasions, 2 days apart. On the first visit, subject characteristics were documented by a general questionnaire on upper and lower respiratory symptoms, history of atopy, asthma, and medications required for NP and/or asthma. Skin prick tests for common inhalant allergens (house dust mite, animal danders, grass pollen, tree pollen, and molds) and spirometric measurements were performed. Serum total IgE was measured by radioimmunosorbent test (Pharmacia Diagnostics, Uppsala, Sweden). On the second visit, subjects underwent methacholine inhalation challenge and, at least 3 hours later, a fiberoptic bronchoscopy for bronchial lavage and bronchial biopsy.

Definitions

Asthma was defined according to the criteria suggested by the American Thoracic Society.¹⁹ The asthma severity was assessed according to Aas²⁰ scoring system (from score 1 for very mild forms to score 5 for an incapacitating disease). Exclusion criteria for patients with asthma were lower respiratory tract infection in the previous 8 weeks, current smoking habit, asthma exacerbation in the preceding 8 weeks, and treatment with inhaled or oral corticosteroids in the 4 months before the study. Asymptomatic BHR was defined as a methacholine PD₂₀ of less than 2550 μ g in the absence of symptoms suggestive of asthma (intermittent wheeze, chest tightness, dyspnea, recurrent cough) in subjects who did not report previous history of childhood asthma and who never required any asthma medication. Atopy was defined as the presence of at least 1 pos-

itive skin prick test response (ie, wheal size superior to that induced by histamine).

Pulmonary function tests

All measurements were made with the patient in the sitting position. FEV₁, forced vital capacity (FVC), and maximal midexpiratory flow (FEF₂₅₋₇₅) were obtained from flow-volume curves with a spirometer (Medgraphics, St Paul, Minn). All measurements were recorded before fiberoptic bronchoscopy. The largest values of FVC, FEV₁, and FEF₂₅₋₇₅ from the first 3 technically satisfactory forced expirations were selected. All data were expressed in absolute values and in percent of predicted normal values.²¹

Methacholine inhalation test

Methacholine inhalation tests were performed according to the guidelines of the European Society for Clinical Respiratory Physiology.^{21,22} They were performed at least 3 hours before fiberoptic bronchoscopy. Previous reports have demonstrated that methacholine inhalation does not alter the airway cellularity in healthy and in asthmatic subjects.^{23,24}

Patients were required to discontinue β_2 -agonists 12 hours before the methacholine challenge was performed. Methacholine challenges were performed sequentially with a dosimeter (MEFAR, Paris, France). Fresh methacholine solutions with concentrations of 1 mg/mL and 10 mg/mL were prepared with methacholine chloride (Allerbio). Patients inhaled from functional residual capacity to total lung capacity, first the diluent solution followed by doubling doses of methacholine, starting from 10 μ g. Doses were given at 5-minute intervals. The procedure was ended when FEV₁ decreased by 20% from postdiluent value or when a cumulative dose of 2550 μ g of methacholine was reached. After each inhalation, the lowest, technically satisfactory FEV₁ value was used in the analysis to calculate the dose-response curve. Dose-response curves were constructed by plotting the FEV₁ against increasing doses of methacholine. The provocation dose (PD₂₀) was defined as the cumulative dose of methacholine necessary to decrease FEV₁ by 20% from baseline values. BHR was defined by a PD₂₀ of less than 2550 μ g.

Assessment of airway inflammation

Fiberoptic bronchoscopy. The bronchoscopy procedure was performed at the same time of day (11 AM). Bronchoscopy was performed according to published guidelines.²⁵ After local anesthesia of the throat, larynx, and bronchi was achieved with 2% lidocaine, a flexible bronchoscope (Olympus OES 10 fiberscope) was introduced into the bronchial tree and gently wedged into a segmental bronchus of the right middle lobe. One 50-mL aliquot of normal saline solution (37°C) was instilled and aspirated with a syringe through the bronchoscope channel. This was referred to as the bronchial lavage (BL). In some patients, biopsy specimens were taken from segmental divisions of the main bronchi after the completion of the lavage. During bronchoscopy, oxygen was readily available, and the patient had an intravenous infusion to provide venous access.

Examination of BL fluid. BL was filtered through sterile surgical gauze to remove mucus. The cells from BL were pelleted by centrifugation at 200g for 10 minutes at 4°C. The supernatant was decanted and stored at -70°C for later analysis. The cells were resuspended in RPMI 1640 and kept on ice until processed. Lavage fluid (10 μ L) was used to determine the total cell count with the use of a standard hemacytometer. Aliquots of the BL (200 μ L) were spun into a cytocentrifuge and stained with eosin/methylene blue (RAL, Paris, France) for differential cell counts. At least 400 cells on each slide were read by 2 investigators who were blinded to the clinical details of the patients. Averaged cell differentials of the 2 investigators are reported. Macrophages, neutrophils, lymphocytes,

eosinophils, mast cells, and epithelial cells were enumerated; results were given both in percentage and number of cells per millimeter of fluid recovered.

Eosinophil cationic protein (ECP) and tryptase were assayed in BL supernatants in duplicate with specific and sensitive radioimmunoassays (Pharmacia, St Quentin Yvelines, France). Histamine was measured in BL fluid supernatant by ELISA (Immunotech, Marseille, France). Results were expressed as micrograms per milliliter for ECP and nanometers for histamine and tryptase.

Biopsy sampling and processing. One bronchial biopsy specimen was fixed in 4% formaldehyde, dehydrated, and embedded in paraffin. The other biopsy specimen was placed in PBS and embedded in optimal cutting temperature medium, snap frozen in isopentane, precooled in liquid nitrogen, and stored at -80°C for immunohistochemistry. Serial sections 6 μm thick were obtained from biopsy specimens. Lymphocytes were evaluated on the paraffin-embedded sections stained with May-Grünwald-Giemsa. Indirect immunoenzymatic methods were used to identify eosinophils and mast cells with mAbs directed against the eosinophil peroxidase (Oncogene Science Inc, Uniondale, NY) and the human tryptase (AA1, Dako, Denmark), respectively. Monoclonal antibody binding was detected with the alkaline phosphatase method (Dako). For each section, the numbers of positively stained cells were counted in several nonoverlapping high-power fields until the available area from the reticular basement membrane to a depth of 100 μm was covered. The results were expressed as the number of cells per square millimeter. To avoid observer bias, the slides were coded before analysis and read blind.

Statistical analysis

Group data are expressed as means $\pm$ SEM. Because most of the data were not normally distributed, statistical analysis was performed with the nonparametric Kruskal-Wallis test for differences among the groups. When the results of this test indicated significant difference, each pairing was examined by means of the Mann-Whitney *U* test. Differences in sex, atopy, and aspirin intolerance were examined by Fisher's exact test. The correlations were investigated with the Spearman's rank correlation coefficient test. A probability value (*P*) of less than .05 was taken as statistically significant.

RESULTS

Clinical data

We studied 35 patients with NP. Characteristics of patients are shown in Table I. According to clinical history and bronchial response to methacholine inhalation challenge, 3 groups of patients were identified: 14 patients did not exhibit BHR (group A); 7 patients with NP had asymptomatic BHR (group B); and 14 patients with NP exhibited BHR associated with asthma (group C).

The 3 groups were not different in regard to age and sex. Serum total IgE level was similar in the 3 groups. Prevalence of atopy and aspirin intolerance tended to be higher in group C than in group A and group B, but this difference did not reach significance. In group C, severity of asthma was mild; asthmatic patients were classified by Aas score 1 (*n* = 9) or 2 (*n* = 5). Asthma symptoms started before the onset of NP in 5 cases, asthma and NP symptoms appeared at the same period in 4 cases, and asthma symptoms appeared after the onset of NP in 5 cases. Nine patients with asthma were using inhaled β_2 -agonists as needed.

Characteristics of NP are summarized in Table II. The

TABLE I. Patient characteristics

	NP without BHR (group A)	NP with asymptomatic BHR (group B)	NP with BHR and asthma (group C)
Patients (n)	14	7	14
Age (y)	42.3 $\pm$ 3.4	37.1 $\pm$ 4.2	39.4 $\pm$ 2.7
Sex ratio (M/F)	10/4	5/2	5/9
Atopy*	1	1	5
IgE (kU/L)	113.8 $\pm$ 41.0	41.6 $\pm$ 15.6	124.6 $\pm$ 36.5
Aspirin intolerance (n)	1	1	5
Asthma (n)	—	—	14
Onset of asthma symptoms			
Before NP (n)	—	—	5
Concomitant to NP (n)	—	—	4
After NP (n)	—	—	5
Daily therapy for asthma (n)	—	—	9†

Data are expressed as means $\pm$ SEM.

*Positive skin prick test responses to 2 or more inhalant antigens.

†Inhaled salbutamol as needed.

nasal score was similar in the 3 groups. Duration of nasal symptoms tended to be longer in group C than in group A and group B, but this difference did not reach statistical significance. The number of patients with a history of polypectomy was similar in the 3 groups. Mean daily dosage of topical steroids for NP was similar in the 3 groups.

Physiologic parameters

Patients with asymptomatic BHR (group B) did not significantly differ from patients without BHR (group A) with regard to FEV₁, FEF₂₅₋₇₅, and FEV₁/FVC ratio (Table III). Patients with asthma (group C) exhibited a lower FEV₁, FEF₂₅₋₇₅, and FEV₁/FVC ratio than patients in group A (*P* < .01). FEV₁ (L) and FEF₂₅₋₇₅ were lower in group C than in group B (*P* < .05), whereas FEV₁ (percent of predicted value) and FEV₁/FVC ratio did not differ significantly between group B and group C. FVC did not differ among the 3 groups. Methacholine PD₂₀ ranged between 80 and 320 μg in group B and between 9 and 800 μg in group C. PD₂₀ was greater in group C than in group B (*P* < .05).

Differential cell counts in BL and in bronchial submucosa

BL was obtained in all cases (*n* = 35). Satisfactory endobronchial biopsy specimens were obtained in 20 patients (7 specimens in group A, 5 specimens in group B, and 8 specimens in group C). In 6 cases, the quality of the specimens did not allow satisfactory analysis of bronchial tissue (3 specimens in group A, 1 specimen in group B, and 2 specimens in group C). In 9 cases, fiberoptic bronchoscopy did not allow the performance of both BL and bronchial biopsy (4 cases in group A, 1 case in group B, and 4 cases in group C) because the patients experienced cough after BL, which was

TABLE II. Characteristics of NP

	NP without BHR (group A)	NP with asymptomatic BHR (group B)	NP with BHR and asthma (group C)	P value*
Nasal score†	6.8 ± 1.7	6.6 ± 2.1	11.9 ± 2.5	.53
Duration of nasal symptoms (mo)	82.1 ± 20.6	78.8 ± 25.8	143.1 ± 29.7	.24
Polypectomy				
Unique (n)	1	0	0	.52
Multiple (n)	2	0	2	
Dosage of topic steroids (µg/d)	533 ± 66	650 ± 50	511 ± 59	.47

Data are expressed as means ± SEM.

*Probability value of Kruskal-Wallis test for quantitative data and Fisher's exact test for qualitative data ($P < .05$ was taken as statistically significant).

†Nasal score included the assessment of nasal obstruction, anosmia, sneezing, rhinorrhea, and itching. Each nasal symptom was scored from 0 to 3 (0 = no symptom; 1 = mild symptom; 2 = moderate symptom; 3 = severe symptom) so that the maximal nasal score was 15 of 15.

TABLE III. Pulmonary characteristics of patients with NP

	NP without BHR (group A)	NP with asymptomatic BHR (group B)	NP with BHR and asthma (group C)	P value*
FEV ₁ (L)	3.75 ± 0.26	3.61 ± 0.22	2.75 ± 0.12†	.003
FEV ₁ (%)	103.8 ± 3.0	98.6 ± 2.8	88.2 ± 3.8‡	.020
FEF ₂₅₋₇₅ (L)	4.16 ± 0.36	3.84 ± 0.46	2.32 ± 0.22§	.002
FEF ₂₅₋₇₅ (%)	95.8 ± 6.3	85.0 ± 8.2	59.1 ± 5.5§	.002
FVC (L)	4.62 ± 0.29	4.45 ± 0.23	3.85 ± 0.18	.13
FVC (%)	103.8 ± 3.1	104.1 ± 3.3	103.9 ± 3.6	.97
FEV ₁ /FVC ratio	100 ± 3	93 ± 2	85 ± 4‡	.008
PD ₂₀ (µg)	—	169.1 ± 31.8	144.7 ± 69.8¶	—

Data are expressed as means ± SEM.

*Probability value of Kruskal-Wallis test for quantitative data ($P < .05$ was taken as statistically significant).

Probability value of Mann-Whitney U test: † $P < .01$, different from groups A and B; ‡ $P < .01$, different from group A; § $P < .001$, different from group A; || $P < .05$, different from group B; ¶ $P < .05$, different from group B.

spontaneously reversible 5 minutes after the bronchoscopy.

BL. The total and differential cell counts in the 35 BLs are reported in Table IV. There was no difference between groups in BL recovery (data not shown). There was no difference among the 3 groups in the number of total cells in BL. The percentage of macrophages, neutrophils, lymphocytes, mast cells, and epithelial cells in each group was not statistically different. The mean percentage of eosinophils and the mean number of eosinophils ($P < .05$) in groups B and C were significantly greater than in group A. The mean number and percentage of eosinophils were not different in group B and in group C. Although this difference did not reach statistical significance, ECP levels tended to be higher in the BL in groups B and C than in group A. Histamine levels did not differ among the groups, although they tended to be greater in group C. Tryptase was similar in the groups.

Similar results were obtained when considering only BL from the 20 patients who underwent both BL and bronchial biopsy during bronchoscopy. The mean percentage of eosinophils in group B ($8.4\% \pm 4.8\%$; $n = 5$) and in group C ($10.7\% \pm 4.6\%$; $n = 8$) was significantly higher than that found in group A ($0.6\% \pm 0.3\%$; $n = 7$; $P < .02$), as was the number of eosinophils ($P < .02$). The percentage of macrophages, neutrophils, lymphocytes,

mast cells, epithelial cells, and ECP, histamine, and tryptase levels (data not shown) did not differ among the 3 groups.

BL characteristics from patients with asthma and aspirin intolerance were similar to those from patients with asthma without aspirin intolerance, with regard to total and differential cell counts, and ECP, histamine, and tryptase levels in BL.

Bronchial biopsy specimens. Pathologic analysis of bronchial biopsy specimens demonstrated that the number of eosinophils in bronchial submucosa in patients in groups B and C was significantly increased compared with patients in group A ($P < .01$; Fig 1). The number of lymphocytes was also greater in groups B and C than in group A ($P < .05$). The number of mast cells was similar in the 3 groups. The number of eosinophils and lymphocytes in bronchial submucosa was similar in asthmatic patients with aspirin intolerance and in asthmatic patients without aspirin intolerance.

Correlations

Eosinophil number in the 35 BLs negatively correlated with FEV₁/FVC ratio ($Rho = -0.34$; $P < .049$). A similar trend was observed between eosinophil percentage and FEV₁/FVC ratio, but the coefficients of correlations were not significant ($Rho = -0.30$; $P = .076$). No signifi-

TABLE IV. Total and differential cell counts in BL in patients with NP

	NP without BHR (group A)	NP with asymptomatic BHR (group B)	NP with BHR and asthma (group C)	P value*
Total cell count (10 ³ /mL)	79.19 ± 10.22	78.61 ± 17.55	61.43 ± 7.11	.44
Macrophages				
Percent	65.4 ± 5.1	66.2 ± 8.7	64.2 ± 6.5	.99
10 ³ /mL	57.67 ± 8.41	53.29 ± 17.33	41.51 ± 7.75	.37
Lymphocytes				
Percent	7.3 ± 1.5	4.7 ± 0.8	6.1 ± 1.5	.63
10 ³ /mL	6.09 ± 2.04	3.48 ± 1.14	3.34 ± 0.72	.57
Eosinophils				
Percent	0.8 ± 0.3	6.4 ± 3.6 [†]	7.1 ± 3.0 [†]	.02
10 ³ /mL	0.85 ± 0.35	5.28 ± 3.00 [†]	4.76 ± 2.17 [†]	.03
Neutrophils				
Percent	6.3 ± 1.7	10.6 ± 2.7	3.9 ± 0.9	.10
10 ³ /mL	3.19 ± 0.68	5.61 ± 1.78	2.09 ± 0.49	.12
Mast cells (%)	0	0	0	—
Epithelial cells				
Percent	19.8 ± 4.0	12.0 ± 5.7	18.7 ± 5.8	.33
10 ³ /mL	12.32 ± 2.49	9.26 ± 4.10	9.16 ± 2.37	.54
ECP (μg/mL)	5.6 ± 1.9	10.1 ± 5.5	11.4 ± 3.8	.51
Histamine (nM)	6.3 ± 2.2	7.6 ± 3.5	14.1 ± 5.4	.47
Tryptase (nM)	2.9 ± 0.4	2.7 ± 0.1	2.9 ± 0.3	.30

Data are expressed as means ± SEM.

*Probability value of Kruskal-Wallis test ($P < .05$ was taken as statistically significant).

Probability value of Mann-Whitney U test: [†] $P < .05$, different from group A.

cant correlation between PD₂₀ and eosinophils in the 35 BLs was disclosed.

Submucosal eosinophils and FEV₁ (percent of predicted value) negatively correlated ($Rho = -0.55$; $P < .028$). FEF₂₅₋₇₅ (percent of predicted value) inversely correlated with eosinophil numbers ($Rho = -0.56$; $P < .025$) and with lymphocyte numbers ($Rho = -0.55$; $P < .03$). There was no relationship between PD₂₀ and eosinophil numbers nor between PD₂₀ and lymphocyte numbers.

DISCUSSION

In the present study, we have demonstrated the presence of bronchial inflammatory changes in patients with NP. We reported an increased number of eosinophils in BL fluids and in bronchial submucosa in subjects with NP and asymptomatic BHR and in subjects with NP and asthma. We also demonstrated an inverse correlation between eosinophil numbers in BL and FEV₁/FVC ratio and between submucosal eosinophil numbers and FEV₁. On the other hand, asymptomatic patients with NP without BHR did not feature eosinophilic lower airways inflammation, despite the nasal pathologic evidence. Our findings demonstrate that eosinophilic inflammation occurs in BHR associated with NP in the absence of overt clinical asthma. However, the factors that determine the occurrence of asthma during the course of NP remain to be documented.

This is the first study to examine the bronchial changes that may be associated with NP and to correlate these find-

ings with the assessment of BHR and/or asthma. Many studies have described an increased prevalence of asymptomatic BHR and/or asthma in association with upper airways disorders (UAD), such as allergic rhinitis,²⁶ acute sinusitis,²⁷ and NARES,¹⁸ as compared with the general population. The prevalence of asthma and asymptomatic BHR in subjects with NP is especially high. Asthma was reported at an average frequency of 29.9% in patients with NP referred for ethmoidectomy and in about 70% of those patients referred to allergy clinics.⁴ Subjects with NP and aspirin intolerance have an even higher association with asthma than subjects with NP alone.⁴ The frequency of nonspecific BHR in nonasthmatic patients with NP was reported with widely disparate data. Positive responses to methacholine provocation were reported in 7 of 15 patients and in 11 of 19 patients with NP without asthma, respectively, by Miles-Lawrence et al⁷ and by Jacobs et al,⁸ whereas Downing et al⁹ did not show evidence of asymptomatic BHR in 13 patients with NP. It may be argued that nasal steroid therapy taken by our patients with NP leads to underestimation of the prevalence and/or the severity of BHR, as it was reported by Watson et al in subjects with allergic rhinitis.²⁸ Nevertheless, in a previous prospective study, we clearly demonstrated that nasal steroid therapy did not affect nonspecific BHR in patients with NP because it remained stable over 12 months.¹⁰ Taken together, these studies displayed evidence of heightened frequency of positive methacholine or carbachol challenges in nonasthmatic subjects with NP. This point is of importance because BHR may be considered a risk factor for the development of asthma.

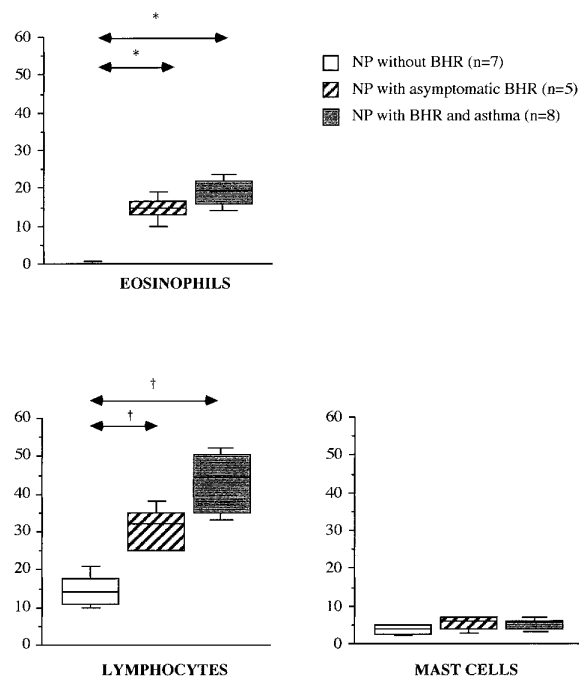


FIG 1. Eosinophils, total lymphocytes, and mast cells in the bronchial submucosa in patients with NP. Data are presented as box plots, and results are expressed as number of cells/mm². Horizontal lines represent the median, squares represent the 25th and 75th percentiles, and bars represent the 10th and 90th percentiles. Probability value of Mann-Whitney *U* test: **P* < .01; †*P* < .05.

Not all patients with NP seem to be prone to the development of nonspecific BHR. Mechanisms connecting upper and lower airway dysfunction in NP are poorly understood. Several theories have been proposed to explain the apparent relationship between nasal diseases and lower airways dysfunction.²⁶ These theories include the activation of nasobronchial reflexes and pulmonary aspiration of nasal contents. We did not assess nasal inflammation in our patients with NP. This was previously done by nasal lavages in patients with NARES, which may be regarded as a precursor of NP in some cases.¹⁸ The authors did not report any correlation between nasal inflammatory contents and the existence of nonspecific BHR. Previous reports have also suggested that preferential mouth breathing in subjects with allergic rhinitis may lead to the development of BHR and asthma because it enhances direct lower airway exposure to airborne allergens and pollutants.²⁶ Mouth breathing as the result of nasal obstruction is a common feature in subjects with NP. However, characteristics of NP in our patients with asymptomatic BHR and in patients without BHR were quite similar in terms of clinical nasal score, previous history of polypectomy, and mean daily dosage of topical steroids. In addition, nasal obstruction score was similar in the 2 groups of patients (data not shown). This point suggests that the characteristics and the severity of NP are not the main factors that determine the occurrence of BHR in NP. It may be hypothesized that additional

factors, such as genetic predisposing conditions, are necessary for BHR to develop in individuals with NP. To summarize, NP is not sufficient per se to induce BHR and lower airways inflammation in all patients.

Few studies have evaluated the pathologic lesions that underlie asymptomatic BHR. In healthy subjects with no previous history of asthma and/or rhinitis, asymptomatic BHR does not seem to be associated with significant inflammatory bronchial changes. Power et al¹⁵ found no differences in the numbers of eosinophils, neutrophils, T cells, and mast cells in bronchial biopsy specimens of asymptomatic healthy subjects with or without BHR. In the same line, Pin et al¹⁶ reported no difference in terms of sputum differential cell counts between children with asymptomatic BHR and asymptomatic normoresponsive children. On the other hand, recent studies have provided evidence in favor of the presence of subclinical bronchial inflammation responsible for BHR in UAD. Some researchers have demonstrated the presence of inflammatory cells, such as eosinophils and mast cells, and bronchial subepithelial fibrosis in bronchial biopsy specimens of subjects with allergic rhinitis.²⁹⁻³¹ More recently, Foresi et al¹⁷ reported an increased number of eosinophils and metachromatic cells in induced sputum of subjects with allergic rhinitis and BHR as compared with subjects with allergic rhinitis without BHR. Moreover, a significant correlation was found between methacholine PD₂₀ and eosinophils and mast cells. Inflammatory bronchial changes occurring during NARES were recently described.¹⁸ The presence of BHR in subjects with NARES was associated with an increased number of eosinophils in induced sputum as compared with subjects with NARES without BHR. Our findings in NP are in keeping with these studies. Indeed, we found that BHR in nonasthmatic patients with NP was associated with an eosinophilic bronchial inflammation and an increased number of submucosal lymphocytes. This bronchial inflammatory pattern was similar to that observed in asthmatic patients with NP. All these findings support the hypothesis of a subclinical bronchial inflammation associated with asymptomatic BHR in subjects with allergic and nonallergic UAD.

Characteristics of BLs and bronchial biopsy specimens of subjects with NP and asthma associated with aspirin intolerance or tolerance are consistent with previous reports. Several investigators have provided convincing evidence that allergic and nonallergic asthma were characterized by an infiltrate of eosinophils and lymphocytes in bronchial epithelium and in lamina propria.^{12,13} Many authors³²⁻³⁴ have also reported an increased proportion of eosinophils and eosinophil mediators, such as ECP and major basic protein, in bronchoalveolar lavage fluids of asthmatic patients. In the present study, we reported similar bronchial inflammatory patterns in patients with NP and asymptomatic BHR and in patients with NP and asthma. This finding raised the question about the contribution of these bronchial inflammatory changes to the pathogenesis of asthma associated with NP. In most studies concerning asthma, the number of

eosinophils in bronchoalveolar lavage and in bronchial biopsy specimens usually correlates with bronchial hyperreactivity levels and disease activity or severity, although such correlation was not noted by Adelroth et al³³ nor by Chan-Yeung et al.³⁴ We found that asthmatic patients with NP exhibited significantly lower methacholine PD₂₀ and FEV₁ (L) than patients with NP and asymptomatic BHR. Although the presence of nonspecific BHR was closely related to an increased number of bronchial eosinophils, we were unable to demonstrate a significant correlation between PD₂₀ and eosinophil numbers in BLs and in bronchial biopsy specimens when the subjects with NP and BHR were considered. Similar results were reported in patients with NARES.¹⁸ On the other hand, we showed that the eosinophilic inflammation was associated with an obstructive ventilatory pattern, suggesting that airway eosinophilic infiltration is a necessary condition for BHR and subclinical airflow obstruction to develop, although it is not the sole event that accounts for the development of asthma in subjects with NP.

It can be assumed that asymptomatic BHR in subjects with NP represents a latent phase of asthma that becomes clinically active over the course of time. This is suggested by the mean duration of the nasal disease, which tended to be longer in subjects with NP and asthma than in subjects with asymptomatic BHR. Previous prospective investigations have reported that asymptomatic BHR may represent an indication of potential asthma in the general population as well as in patients with allergic rhinitis.³⁵⁻³⁷ The reasons that asymptomatic BHR does or does not translate into asthma are unclear. The role of atopy as a risk factor to the development of asthma has been proposed by Laprise and Boulet,³⁷ who reported the outcome of asymptomatic BHR in 30 subjects over a 3-year prospective follow-up. We also reported that the prevalence of atopy tended to be higher in patients with asthma than in patients with asymptomatic BHR, although this difference did not reach statistical significance. This point needs to be further confirmed in a greater number of subjects with NP. Indeed, it would be of interest to determine which subjects with NP and asymptomatic BHR are at greater risk for the development of clinical asthma. Increased nonspecific BHR, obstructive ventilatory pattern, and atopy may represent indicators or risk factors for the development of subsequent asthma in subjects with NP. It is likely that other factors (aspirin intolerance) are involved in the development of asthma.

In conclusion, we have demonstrated the existence of subclinical bronchial changes in patients with NP and asymptomatic BHR. These changes were similar to those observed in patients with NP and clinical asthma, whereas patients with NP without BHR did not feature lower airways inflammation. Although the eosinophilic airway infiltration was closely associated with the existence of BHR, its contribution to the development of asthma is uncertain. Asymptomatic BHR associated with NP may represent an intermediate stage before the development of asthma. The mechanisms by which asymptomatic

BHR does or does not translate into asthma remain to be documented. Further prospective studies are needed to assess the outcome of asymptomatic BHR in patients with NP and to identify which subjects with NP are at risk for the development of overtly symptomatic asthma.

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