

Original article

Airway inflammation in asthma and perennial allergic rhinitis. Relationship with nonspecific bronchial responsiveness and maximal airway narrowing

Background: Eosinophilic airway inflammation is the hallmark of asthma, but it has also been reported in other conditions such as allergic rhinitis. We have tested whether the analysis of cells and chemicals in sputum can distinguish between patients with mild allergic asthma, those with allergic rhinitis, and healthy controls. The relationship between inflammation markers in sputum and nonspecific bronchial hyperresponsiveness to methacholine (BHR) (PD₂₀ and maximal response plateau [MRP] values) was also evaluated.

Methods: We selected 31 mild asthmatics and 15 rhinitis patients sensitized to house-dust mite. As a control group, we studied 10 healthy subjects. Every subject underwent the methacholine bronchial provocation test (M-BPT) and sputum induction. Blood eosinophils and serum ECP levels were measured. Sputum cell differentials were assessed, and eosinophil cationic protein (ECP), tryptase, albumin, and interleukin (IL)-5 levels were measured in the entire sputum supernatant.

Results: Blood eosinophils and serum ECP levels were higher in asthma patients and rhinitis than in healthy controls, but no difference between asthma patients and rhinitis patients was found. Asthmatics had higher eosinophil counts and higher ECP and tryptase levels in sputum than rhinitis patients or control subjects. Sputum albumin levels were higher in asthmatics than in controls. Rhinitis patients exhibited higher sputum eosinophils than healthy controls. An association between sputum eosinophil numbers and MPR values ($r = -0.57$) was detected, and a trend toward correlation between sputum ECP levels and PD₂₀ values ($r = -0.47$) was found in the rhinitis group, but not in asthmatics. No correlation between blood eosinophilic inflammation and lung functional indices was found.

Conclusions: Induced sputum is an accurate method to study bronchial inflammation, allowing one to distinguish between rhinitis patients and mildly asthmatic patients. The fact that no relationship was detected between sputum inflammation and BHR suggests that other factors, such as airway remodeling, may be at least partly responsible for BHR in asthma.

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Prominent infiltration of the airway by activated eosinophils (1) and nonspecific bronchial hyperresponsiveness (BHR) (2) are constant findings of asthma. Several attempts have been made to demonstrate a causative relationship between both conditions. The results, however, have been contradictory, since some authors have reported a relationship between BHR and eosinophilic inflammation (3), but others have not detected such an association (1).

The mechanisms inducing a selective recruitment of eosinophils to the airways are not well known. There is increasing evidence that the TH₂-cell subset, through the release of substances that selectively prime eosino-

phils, such as interleukin (IL)-5, or that enhance IgE-mediated responses (IL-4), plays a central role in the onset of bronchial inflammation (4). On the other hand, mast cells, which have been implicated in acute asthma, can also release a cytokine profile very similar to that described for TH₂ cells (5). Mast cells can also produce other substances, such as tumor necrosis factor (TNF)- α or tryptase, that are involved in the chronic changes of the airway (5). Taken together, these data suggest that, mainly in the early stages of asthma, mast cells might play a role in the onset of airway inflammation (5).

Airway inflammation has been studied by indirect

methods (peripheral blood) or invasive, fibrobronchoscopy-derived direct techniques. The induced sputum technique (6) and the treatment of the sample with cell-dispersion reagents (7) has proved to be a reproducible (6, 8–10) and valid (11–14) method to measure airway inflammation directly and noninvasively.

Although allergic rhinitis is probably a risk factor for asthma development, the relationship between the two disorders is not clear (15). A widely overlapping degree in sensitivity to methacholine has been reported between rhinitis patients and asthma patients (2). Therefore, it has been suggested that the identification of a maximal response plateau (MRP) might be a more discriminative index for differentiation of the two clinical conditions (16). Eosinophilic mucosal infiltration has also been reported in bronchial biopsies from allergic rhinitis patients (3, 17). Recently, Foresi et al. (18) have shown that induced sputum from seasonal allergic rhinitis patients exhibited an intermediate degree of eosinophilic inflammation, halfway between asthma and healthy controls, and that levels of sputum eosinophils in rhinitis patients were higher in those who also presented BHR (18). Furthermore, allergen inhalation by sensitized rhinitis patients could elicit an asthma-like eosinophilic response in bronchoalveolar lavage (BAL) (19). Taken together, these data suggest the lack of a clear border between allergic rhinitis and asthma.

The aim of our study was firstly to analyze the differences between cells and chemicals measured in induced sputum and blood from asthmatic patients and rhinitis patients who were sensitized to *Dermatophagoides pteronyssinus*, and from healthy subjects. Secondly, we aimed to assess whether inflammatory markers in sputum could distinguish between allergic asthma and rhinitis better than when measured in blood. Thirdly, we tried to determine whether sputum inflammatory markers were correlated to the baseline lung function and to the degree of BHR determined by the complete dose-response curves in which the MRP was examined.

Material and methods

Subjects

Thirty-one mildly asthmatic patients and 15 rhinitis patients sensitized to *D. pteronyssinus* were selected. We also studied 10 nonatopic healthy subjects as a control group. In accordance with international guidelines (20), the diagnosis of asthma was based on a clinical history of recurrent attacks of wheezing, cough, breathlessness, and chest tightness. Only short-acting β -agonists used “as needed” were allowed in the previous 2 months. Patients having a clinical history of nonseasonal rhinitis in the absence of asthma symptoms (cough, wheezing, dyspnea, and chest tightness) were matched as the

“rhinitis group”. A history of respiratory symptoms lasting 12–24 months was required in both groups. Patient selection was not carried out according to the presence of BHR. No patient had a history of smoking, had suffered from lower or upper respiratory tract infections during the previous 2 months, or had been treated with corticosteroids within the preceding 2 months.

Sensitization to *D. pteronyssinus* was established by positive skin prick tests (SPT) with a standardized extract (Abelló, Madrid, Spain) and specific IgE (Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden) to this allergen in the context of a compatible clinical history. Patients sensitized to other perennial or seasonal allergens (animal danders, molds, or pollens) were excluded.

All subjects gave informed consent before starting the study. The ethics committee of our hospital approved the study protocol.

Study design

Asthma patients, rhinitis patients, and healthy controls underwent clinical and physical evaluation, and samples of venous blood and induced sputum were collected. Eight hours later, the subjects underwent the methacholine bronchial provocation test (M-BPT), and complete dose-response slopes (DRS) were recorded.

Cutaneous tests

Cutaneous tests were performed by means of SPT. We tested the most common airborne allergens in our region (mites, pollens, molds, and animal danders) and a standardized *D. pteronyssinus* extract (Abelló, Spain). Histamine phosphate at 10 mg/ml and PBS were used as positive and negative controls, respectively. Lancets (Prick-Lancetter, Dome Hollister-Stier Laboratories, UK) were used in the SPT, and the results were read at 15 min. The resulting wheal of the SPT was traced with a thin pen and transferred to millimeter squared paper. The area size was then measured and the result was expressed in mm².

Bronchial hyperresponsiveness (BHR)

To avoid circadian rhythm effect on airway dynamics, every M-BPT was carried out between 2 and 4 pm. Nonspecific BHR was assessed with methacholine (Provocholine, Roche Laboratory, Nutley, NJ, USA) as agonist. Methacholine dilutions at 0.125, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 100.0, and 200.0 mg/ml in PBS were made on the day of the test. Baseline spirometry was performed and forced expiratory volume in 1 s (FEV₁) and FEV₁/VC (vital capacity) index were recorded. The reference values of Crapo et al. (21) were used. FEV₁ values equal to or higher than 70% of predicted normal were required to start the test.

Patients had not inhaled β -agonist drugs for the 8 h preceding the test. Diluent (PBS) and methacholine were administered with a MEFAR dosimeter (MEFAR s.r.l. Borezzo [BS], Italy), programmed to deliver five inhalations of 1 s each. Patients inhaled 10 μ l of solution in each inhalation. The FEV₁ value was measured by spirometry 3 min after inhalation. A variability rate lower than 5% among basal and postdiluent FEV₁ values was required to start the test. Methacholine at increasing concentrations was administered by the dosimeter. The test finished when a fall in FEV₁ values equal to or higher than 40% from the postdiluent value was achieved, or when the highest concentration of methacholine was inhaled. Results were expressed in terms of the M-PD₂₀ (cumulative provocative dose of methacholine, expressed in μ mol [1 mol methacholine=195.4 g], needed to decrease FEV₁ by 20% of the baseline values), the DRS (rate between the maximal FEV₁ fall and the highest dose of agonist inhaled), and the MRP. An MRP was recognized if three or more of the highest doses of agonist fell within a 5% response range. The level of maximal response was obtained by averaging the data points on the plateau. In the absence of a plateau, the largest fall in FEV₁ was documented and not considered for analysis.

Sputum induction

Sputum was induced at least 6 h before the M-BPT in all the patients. Sputum samples were obtained by hypertonic saline inhalation according to Gershman et al. (22) with several modifications. The subjects inhaled four puffs of salbutamol 30 min before sputum induction. To avoid contamination of the sample, subjects were asked to rinse their mouths and blow their noses before induction and, when possible, before expectoration. An ultrasonic nebulizer (Ultraneb 99, De Vilbiss, Somerset, PA, USA) was used to administer saline at 5%, for three periods of 10 min each. After each period, the patient was asked to cough and

expectorate into a sterile container. Meanwhile saliva was, when possible, put into a different recipient. The test finished when a macroscopically adequate sputum sample was obtained or when the three periods of inhalation were completed.

The volume of the entire sputum sample was measured, and 3 ml of the entire sputum sample was mixed with an equal volume of dithiotreitol (DTT) (Sputasol, Unipath, Basingstoke, Hampshire, UK) at 1/100, and rocked at room temperature for 15 min. Then, the mixture was passed through one 0.42- μ m Millipore filter (Millipore, Somerset, PA, USA) and centrifuged at 1500 g for 10 min. The supernatant was then aliquoted and frozen at -70°C until further analysis. The pellet was suspended in saline serum at 0.9%, and total cells were counted with a Fuchs Rosenthal chamber. The suspension was then cytocentrifuged and stained with either Papanicolaou or Giemsa for differential cell counts. The sample was considered adequate for analysis when macrophages could be visualized and squamous cell contamination was lower than 20% (9). Percentual counts of macrophages, eosinophils, neutrophils, mast cells, and lymphocytes were made over a total count of 400 cells. Evaluation was done by a single person, blind to the clinical conditions of the patients.

Soluble markers in sputum and serum

The concentrations of ECP and tryptase in the sputum supernatant, of serum ECP, and of serum total and specific IgE were measured by a fluoroenzyme immunosorbent assay (UniCAP, Pharmacia Diagnostics, Uppsala, Sweden). Sputum IL-5 levels were determined in duplicate by a commercial "sandwich" enzyme-linked immunosorbent assay (ELISA) (Quantikine; R&D Systems, Minneapolis, MN, USA). Sputum albumin levels were measured in duplicate by automated nephelometry (Behring Diagnostics GmbH, Marburg, Germany). The limits of detection for the fluid-phase assays were 0.35 $\mu\text{g/l}$ for ECP and tryptase,

Table 1. Patients' demographic, clinical, and functional respiratory data

<i>n</i>	Asthma 31	Rhinitis 15	Control 10
Sex	17 (55%) male	10 (66%) male	5 (50%) male
Age (years)	21 (IQR: 19–29)	21 (IQR: 18–23)	28 (IQR: 25–32)
Total IgE (kU/l)	174 (IQR: 110–292)	98.4 (IQR: 26–149)	10.9 (IQR: 3.7–43.2)
Specific IgE (kU/l)	44.9 (IQR: 19.2–58)	18.1 (IQR: 6.9–29.3)	Undetectable
Skin test (wheal area [mm ²])	88.5 (IQR: 72–109)	68.9 (IQR: 52–99)	Negative
FEV ₁ (%)	100 (IQR: 94–108)	103 (IQR: 93–108)	110 (IQR: 104–118)
FEV ₁ /VC	0.91 (IQR: 0.86–0.97)	0.98 (IQR: 0.94–0.99)	0.97 (IQR: 0.95–0.99)
PD ₂₀ (μ mol)	4.40 (IQR: 0.86–3.83)	49.64 (IQR: 24–100.8)	Undetectable
Slope (%/ μ mol)	1.78 (IQR: 0.86–3.83)	0.19 (IQR: 0.14–0.28)	0.12 (IQR: 0.06–0.18)
Maximal response plateau (%)	20.0 (IQR: 14.75–29.0)	6.0 (IQR: 2.5–12.9)	10.0 (IQR: 6.0–14.0)

IQR: interquartile range.

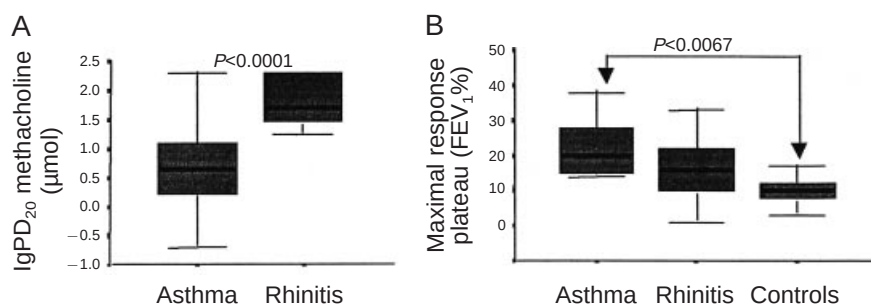


Figure 1. Differences in methacholine PD₂₀ values (A) and maximal response plateau values (B) among asthma patients, rhinitis patients, and control subjects (Mann–Whitney U test). Only patients who presented identifiable methacholine PD₂₀ values or MRP were included for analysis. Methacholine PD₂₀ values were obtained in every asthma patients and in seven out of 15 rhinitis patients. MRP was identified in 10 of asthma patients, in 14 of rhinitis patients, and in every control subject.

3.0 pg/ml for IL-5, and 1 mg/ml for albumin. Results for sputum chemicals were referred to the sputum and DTT mix volume. All assays were performed blind to the clinical details.

Statistical analysis

Statistical analysis of the data was performed by the SPSS Windows 6.0 statistics program. Descriptive statistics were used to summarize the clinical and demographic characteristics of the patients. Results were expressed as median and interquartile rate (IQR). PD₂₀ and DRS values were log-transformed for analysis. Differences between groups were examined by the Kruskal–Wallis analysis of variance. The Mann–Whitney U test was used to evaluate differences between two groups. Correlation between variables was determined by Spearman's rank correlation coefficient. A *P* value of <0.05 was considered significant.

Results

The demographic characteristics, degree of sensitization, and respiratory functional indices of the three groups of subjects are shown in Table 1. The MRP was identified in a lower rate (*P*=0.0003) of asthma (32%) than rhinitis (93%) patients. The Kruskal–Wallis test showed significant differences between groups for FEV₁ (*P*=0.008), FEV₁/VC (*P*=0.023), M-PD₂₀ and DRS (*P*<0.001), and MRP (*P*=0.008). Individual differ-

ences between the three groups of patients are shown in Table 2. Differences in M-PD₂₀ values and MRP values are displayed in Fig. 1.

Cell counts and soluble markers in induced sputum and blood

Blood and sputum samples were obtained from every subject. Total blood eosinophils, and total and differential cell counts in sputum are shown in Table 3. ECP levels in serum and in sputum, and albumin levels in sputum were detected in every sample. Tryptase and IL-5 were assayed in all the asthmatic or control sputum samples and in nine (60%) of the rhinitis sputum samples. Tryptase levels were detected in seven of the asthmatic patients but in no rhinitis patients or control subjects. IL-5 was undetectable in all the sputum samples. Median and IQR values of soluble markers are shown in Table 4.

The Kruskal–Wallis test showed differences among the groups in blood and sputum eosinophils (*P*<0.0001), sputum total cell counts (*P*=0.05), sputum macrophages (*P*=0.034), sputum ECP (*P*=0.001), and sputum tryptase (*P*=0.042). A trend toward significant differences was detected in sputum albumin (*P*=0.084) and serum ECP levels (*P*=0.087). Differences in eosinophilic inflammation among the three groups are shown in Figs. 2 and 3. Eosinophil numbers and ECP levels in blood and sputum were higher in asthmatics than in healthy controls, but only when measured in sputum were these markers higher in asthma than in rhinitis. Eosinophil numbers in blood and sputum and ECP levels in serum were higher in rhinitis patients than controls, but no difference in sputum ECP levels was found between the two groups. Sputum albumin levels were higher in asthmatics than in healthy controls (*P*=0.027), but were similar to those of rhinitis patients.

Table 2. Differences in functional respiratory indices among asthma patients, rhinitis patients, and control subjects (Mann–Whitney U test)

	Asthma/control	Rhinitis/control	Asthma/rhinitis
FEV ₁ (%)	<i>P</i> =0.0004	<i>P</i> =0.013	NS
FEV ₁ /VC	<i>P</i> =0.035	NS	<i>P</i> =0.027
PD ₂₀ (μmol)	<i>P</i> <0.0001	<i>P</i> =0.0055	<i>P</i> <0.0001
DRS (%/μmol)	<i>P</i> <0.0001	<i>P</i> =0.0537	<i>P</i> <0.0001
Maximal response plateau (%)	<i>P</i> =0.0014	<i>P</i> =0.0874	NS

NS: not significant.

Correlations between inflammatory and functional indices

Eosinophils and ECP in sputum correlated in asthma patients (*P*=0.001, *r*=0.57), but not in rhinitis patients. Sputum ECP levels were associated with sputum

Table 3. Median and IQR values of blood (B) eosinophils and total and differential cell counts in sputum (Sp)

	Asthma patients	Rhinitis patients	Controls
B eosinophils (cells/mm ³)	359 (IQR: 233–542)	255 (IQR: 194–490)	100 (IQR: 80–200)
Sp total cell (cells/mm ³)	768 (IQR: 544–1762)	598 (IQR: 133–1109)	202 (IQR: 49–1338)
Sp eosinophils (%)	10.0 (IQR: 3–20)	3.0 (IQR: 0–8)	0 (IQR: 0–0)
Sp neutrophils (%)	22.0 (IQR: 10–34)	11.0 (IQR: 7–31)	19.0 (IQR: 0–37.5)
Sp macrophages (%)	60.0 (IQR: 46–70)	69.0 (IQR: 61–87)	73.5 (IQR: 55–100)
Sp lymphocytes (%)	4.0 (IQR: 2–7)	2.0 (IQR: 1–7)	3.5 (IQR: 0–7)

albumin ($P=0.007$, $r=0.47$) and tryptase ($P=0.039$, $r=0.38$) levels in asthma patients, and with albumin levels ($P=0.003$, $r=0.71$) in rhinitis subjects. The levels of albumin and tryptase in the sputum of asthma patients were also associated ($P<0.001$, $r=0.73$). Sputum eosinophils correlated with blood eosinophils and with serum ECP in both the asthma ($P=0.002$, $r=0.55$ and $P=0.028$, $r=0.39$) and the rhinitis ($P=0.037$, $r=0.54$ and $P=0.038$, $r=0.56$) groups. Sputum ECP levels were associated with blood eosinophils ($P=0.005$, $r=0.50$) in asthma patients. There was an inverse correlation between sputum eosinophils and MRP ($P=0.042$, $r=-0.57$) and a trend toward an association between sputum ECP levels and M-PD₂₀ values ($P=0.075$, $r=-0.47$) in the rhinitis group, but not in the asthma group. In the rhinitis group, sputum albumin levels were associated with M-PD₂₀ values ($P=0.040$, $r=-0.53$). Eosinophilic inflammation in blood showed no relationship with BHR.

Discussion

In this report, we studied a homogeneous group of *D. pteronyssinus*-allergic, untreated, and short-evolution (12–24 months) mildly asthmatic patients. We selected this type of patient, firstly, to avoid as far as possible, the influence of the bronchial remodeling process due to chronic airway inflammation on the results (23); and, secondly, to compare the capacity of induced sputum and peripheral blood to distinguish between patients with mild asthma and those with

allergic rhinitis. Patients were grouped according to an exhaustive clinical history in which we confirmed that rhinitis patients had never experienced bronchial symptoms. In accordance with other reports, our results show higher eosinophil levels and ECP values in asthmatics than healthy subjects, both in blood (24, 25) and in induced sputum (8–10, 12, 26). Eosinophils and ECP values were also higher in sputum from asthma patients than rhinitis patients, but this difference was not observed in peripheral blood.

It has been reported that rhinitis patients exhibit a degree of eosinophilic inflammation both in bronchial biopsies (3, 17) and in BAL (27), but, to date, only two studies have compared eosinophilic inflammation in induced sputum from asthma patients and rhinitis patients (10, 18). In agreement with our results, these studies reported higher percentages of eosinophils and higher levels of ECP in sputum from asthma patients than rhinitis patients. However, when rhinitis subjects were compared to controls, Foresi et al. (18) reported higher eosinophil percentages and ECP levels in the first group, but Spanavello et al. (10) did not find such a difference. In our study, the sputum eosinophil percentage was higher in rhinitis patients than in healthy controls, but we did not find such a difference when we analyzed sputum ECP levels. Our results might be attributed to the dilutional effect that saliva exerts on fluid-phase chemicals when the entire sputum sample is analyzed (12). However, we think that the differences are most likely due to the features of the selected patients: all the rhinitis patients were sensitized to a perennial allergen, a fact that implies a

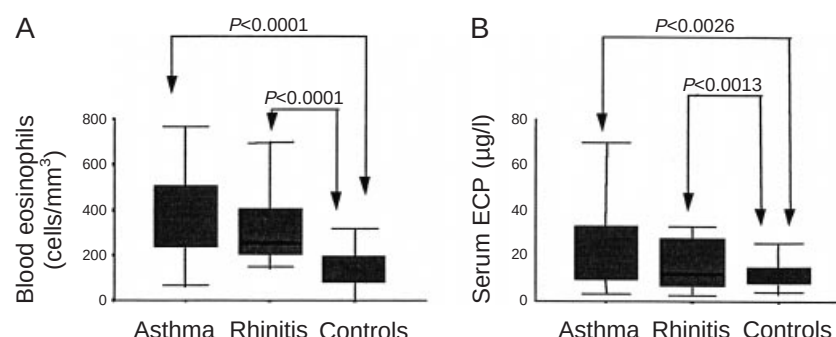


Figure 2. Differences in eosinophil numbers (A) and ECP levels (B) among asthma patients, rhinitis patients, and control subjects, when measured in peripheral blood (Mann–Whitney U test).

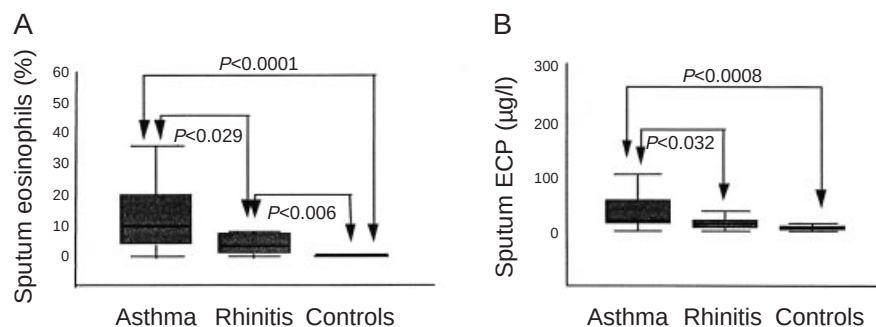


Figure 3. Differences in eosinophil percentage (A) and ECP (B) levels among asthma patients, rhinitis patients, and control subjects, when measured in sputum (Mann–Whitney U test).

continuous stimulus over the airway, and explains the higher eosinophil percentages found. The rhinitis patients had never experienced asthma symptoms, and although PD_{20} values were detectable in 50% of them, these values were much higher than the values reported by Foresi et al. (18). On the other hand, Spanavello et al. selected seasonal allergic rhinitis outside the exposure period, in which BHR was undetected (10). In keeping with their study, our study found that the eosinophil percentage and the ECP levels correlated in sputum from asthmatics, but not in sputum from rhinitis patients. These data strengthen the hypothesis that, although presenting airway eosinophilic infiltration, rhinitis patients have a lower degree of eosinophilic bronchial activation than asthmatic patients.

Although the former results indicate the existence of a relationship between airway eosinophilic activation and BHR, there is no consensus on this point. Some authors have found significant correlations among PD_{20} values and sputum eosinophils (8, 28) or sputum ECP levels (29, 30), whereas other studies have failed to find such a relationship (6, 10, 31, 32). The mechanisms determining the sensitivity (PD_{20}) and the response (MRP) to the agonist are thought to be different (33). However, it is feasible that airway inflammation contributes, at least in part, to both (2): through the release of cationic proteins, eosinophils can damage the respiratory epithelium and increase the airway permeability, enhancing the sensitivity to the agonist.

On the other hand, the infiltration of the bronchial mucosa by inflammatory cells increases the thickness of the bronchial wall, causing a higher airway lumen

stenosis for the same bronchial muscle contraction. ECP can also activate myofibroblasts (34), contributing to the characteristic remodeling process that causes the decrease of the lung elasticity forces (23). Accordingly, we found an association between sputum eosinophil counts and MRP values, and a trend toward correlation between sputum ECP levels and PD_{20} values in rhinitis patients. Such associations were not detected in the asthmatic group, perhaps because a degree of airway remodeling can be found even in mild and short-evolution asthma (1), and it would enhance BHR. Therefore, it is possible that the influence of airway inflammation in BHR is lower in asthma than in rhinitis.

IL-5 is the main cytokine priming eosinophilic chemotaxis and activation (4). The complete lack of detection of IL-5 in our study could be attributed to the dilution of the sputum sample or to a possible DTT-induced denaturalization of the IL-5. The latter contingency is not likely, since other authors have reported that sputum treatment with DTT does not affect IL-5 measurement (8). In accordance with our results, Louis et al. (30) have recently reported the absence of IL-5 in whole-sputum samples, despite strong spiking of the sputum supernatant with cytokines. Therefore, our results could be due not merely to a dilutional effect, but to the binding of cytokines to mucus, an effect that would make detection of IL-5 in the supernatant difficult after filtering of the sample (30).

To evaluate the role of mast cells in the airway inflammation, we measured the levels of tryptase, their specific activation marker. In agreement with other reports (8, 9), sputum tryptase detection was low but selective for asthmatic patients. The degree of

Table 4. Median and IQR values of serum (Se) and sputum (Sp) fluid-phase markers

	Asthma patients	Rhinitis patients	Controls
Se ECP (μg/l)	16.8 (IQR: 8.63–34.0)	11.75 (IQR: 6.1–28.6)	10.15 (IQR: 7.5–15.7)
Sp ECP (μg/l)	19.0 (IQR: 13.0–63.6)	11.8 (IQR: 3.7–20.5)	3.39 (IQR: 1.9–10.17)
Sp albumin (mg/ml)	10.8 (IQR: 5.7–119)	8.3 (IQR: 0–18.2)	5.22 (IQR: 0–8.43)
Sp tryptase (μg/l)	0 (IQR: 0–0.75)	Undetectable	Undetectable
Sp IL-5 (pg/ml)	Undetectable	Undetectable	Undetectable

correlation detected among tryptase, ECP, and albumin in sputum suggested simultaneous activation of eosinophils and mast cells. Tryptase, through the enhancement of smooth-muscle contraction, the activation of collagenase, and the disruption of respiratory epithelium intercellular unions (5), seems to be deeply involved in the bronchial muscle response to agonists, in the airway remodeling process, and in the fragility of the respiratory epithelium. Therefore, we would have expected to find a relationship between levels of tryptase and the MRP values. However, we were unable to detect such an association, a fact that might be attributed to the low number of asthma patients in whom MRP was identified.

In agreement with other reports (8, 9), we found the levels of albumin in sputum from asthma patients to be higher than in healthy controls. When the rhinitis group was analyzed, albumin levels did not differ from those of asthmatics or control subjects. Sputum albumin levels were directly correlated with sputum ECP levels in both groups and with sputum tryptase levels in the asthmatic group, suggesting that activated eosinophils and mast cells, through the release of vasoactive mediators, increase vascular permeability.

In conclusion, we have demonstrated that analysis of the eosinophilic inflammation in sputum samples allows one to distinguish between patients with mild asthma, those with allergic rhinitis, and healthy controls. Perennial allergic rhinitis patients present a degree of eosinophilic inflammation in sputum intermediate between asthma patients and control subjects. The absence of a closer correlation between sputum inflammation and BHR might be due to the multifactorial origin of BHR. Analysis of induced sputum samples reflects the inflammatory changes occurring on the intrapulmonary epithelial lining fluid but gives no information about the chronic changes of the remodeling process that have been established at the airways.

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