

Indirect evidence of bronchial inflammation assessed by titration of inflammatory mediators in BAL fluid of patients with asthma

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Bronchial inflammation is a characteristic of asthma that may be examined indirectly by bronchoalveolar lavage (BAL). Nine normal individuals were compared with 38 age-matched adults with asthma of variable severity to appreciate the importance of cell activation in the severity of asthma. The severity of asthma was appreciated by the clinical score of Aas and the pulmonary function of the patients. FEV₁ ranged between 35% and 130% of predicted. The indirect activation of eosinophils (EOSs), mast cells, fibroblasts, and neutrophils was examined by the titration of eosinophil cationic protein (ECP), tryptase, hyaluronan (HA), and myeloperoxidase (MPO) by radioimmunoassay in BAL fluid (BALF) and cytology of BALF. In the adults with asthma, there was a significantly increased number of EOSs and a significantly increased level of all mediators but MPO. MPO levels were increased in seven patients only; three of these patients were previous smokers. Only ECP and HA levels were significantly correlated with the severity of asthma. These results demonstrate EOSs, mast cells, and fibroblasts are activated in asthma, whereas the involvement of neutrophils is less clear. There was a significant correlation between ECP and HA levels, suggesting a common activation of EOSs and fibroblasts. (J ALLERGY CLIN IMMUNOL 1991;88:649-60.)

Key words: Bronchial inflammation, BAL, mediators, asthma

The major characteristic of asthma is bronchial inflammation leading to nonspecific hyperreactivity¹ that can be assessed directly by pathologic observations and indirectly by examining cells and mediators present in BALF, sputum, and/or peripheral blood.²⁻⁵ Until 1985, most pathologic studies were made on patients who died from asthma attacks⁶⁻¹³ and described most of the pathologic findings of asthma.

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Supported in part by a GRECO (Centre National de la Recherche Scientifique) grant.

Received for publication Sept. 12, 1990.

Revised April 4, 1991.

Accepted for publication May 2, 1991.

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Abbreviations used

BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
ECP:	Eosinophil cationic protein
HA:	Hyaluronan
HABP:	Hyaluronan binding protein
MPO:	Myeloperoxidase
EOS:	Eosinophil
CV:	Coefficient of variation
FEF ₂₅₋₇₅ :	Forced expiratory flow rate between 25% and 75% of FVC
MBP:	Major basic protein
PGD ₂ :	Prostaglandin D ₂

Although these postmortem studies are of great importance and both large and small bronchi can be examined, they cannot be extrapolated for all patients with asthma at least because they are performed on the most severe patients.⁹ Since 1985, bronchial biop-

sies have been undertaken in patients with mild to moderately severe asthma on the large airways by means of fiberoptic bronchoscopy.¹⁴⁻²⁰ These studies demonstrated the importance of EOS inflammation,¹⁹ degranulation of mast cells,¹⁴ shedding of the epithelium, lymphocyte infiltration, and a subepithelial fibrosis of the airways responsible for a pseudo-thickening of the basement membrane.¹⁸ These data are of importance in the understanding of bronchial inflammation, but they only reveal abnormalities of the main bronchi; they cannot properly quantitate the inflammation and do not assess the possible heterogeneity of the lesions at different levels of the bronchial tree. These studies should therefore be supplemented by other methods making it possible to quantitate better the inflammation and study small and large airways.

BAL proposed 10 years ago in patients with asthma²¹ explores ill-defined sites of the lung, including small and large airways, as well as alveoli, and provides useful indirect information on the inflammatory state of the asthmatic airways.^{3, 22-24} This technique made it possible to demonstrate the presence of activated cells, such as macrophages,²¹⁻²⁸ EOSs,^{28, 29} or mast cells,²⁹⁻³¹ and increased levels of inflammatory mediators, such as histamine,^{32, 33} PGD₂,^{33, 34} leukotrienes,³⁵ tryptase,³⁶ or EOS granule-derived proteins^{19, 28, 29} or HA.³⁷ However, most of these studies were performed in patients with mild asthma, and none of the studies correlated cellular or biochemical parameters with the severity of asthma in patients suffering from mild to severe disease.

Bronchial biopsy specimens and BALF studies demonstrated that many cell types are activated in asthma. Some of the cells releasing rather specific mediators include EOSs,* releasing granule constituents, such as ECP^{28, 39, 40}; mast cells,^{7-11, 30-34} releasing tryptase^{36, 41-43} and fibroblasts,¹⁸ releasing HA.⁴⁴ However, this glycosaminoglycan may derive from leakage of lymph.⁴⁵ Although HA levels are very low in BALF of normal subjects, increased levels were observed in lung fibrotic diseases,⁴⁶⁻⁴⁸ in which it is considered as a marker of activated fibroblasts, and in asthma.³⁷ MPO is a specific marker of neutrophils stored in the azurophil granule and released during phagocytosis or cell activation.⁴⁹ In contrast, there is no specific marker of alveolar macrophages fully accepted.

In the present study, the indirect activation of EOSs, mast cells, fibroblasts, and neutrophils was examined by means of the titration of ECP, tryptase, HA, and

MPO in BALF of nine normal individuals and 38 adult patients suffering from asthma of variable severity in an attempt to (1) appreciate the importance of EOS, mast cell, fibroblast, and neutrophil activation in asthma, depending on the severity of the disease, and (2) estimate if EOSs and/or mast cells may be involved in the activation of fibroblasts as has been suggested *in vitro*.

MATERIAL AND METHODS

Subjects

We studied 38 patients with asthma (aged 19 to 59 years; mean $\pm$ SD, 37.8 $\pm$ 12.6 years). Asthma was defined according to the criteria of the American Thoracic Society,⁵⁰ and all patients had either a reversible airway obstruction characterized by an increase of 15% of FEV₁ after inhalation of 200 μ g of salbutamol or a positive inhalation challenge with carbachol.⁵¹ The carbachol inhalation challenge was performed at least 2 weeks before bronchoscopy in patients who had a baseline FEV₁ >80% of predicted values.⁵² None of the subject had smoked within the previous 2 years, and none but three subjects had smoked more than five annual packs. No subject had any bronchial or respiratory tract infection during the month preceding the test. Patients were excluded from the study if they had had a severe exacerbation of asthma requiring hospitalization during the month preceding the study or if they had taken systemic corticosteroids of any form during the past 2 months, inhaled corticosteroids the previous month, had taken nedocromil sodium, sodium cromoglycate, or ketotifen the week before the study and theophylline in the previous 48 hours. β_2 Agonists were only withdrawn for 8 hours.

Nine normal healthy volunteers (22 to 57 years of age; mean $\pm$ SD, 37.2 $\pm$ 11.1 years) were used as a control group. Their pulmonary function was within normal range. They were nonallergic and had never suffered from asthma. None of the subjects were current or previous smokers. No subject had any bronchial or respiratory tract infection during the month preceding the test.

The study was done after informed consent and approval of the study by the ethic committee of the hospital.

Investigations: Asthma score

The clinical severity of asthma was quoted according to the score of Aas⁵³ used to grade chronic asthma from very mild forms (score of 1) to incapacitating disease requiring medications (score of 5). The grading is based on events that took place during a year and combines symptoms (number and duration of asthma episodes, total duration of symptoms, and presence or absence of symptom-free intervals between attacks) and the requirements for medications. It does not take into account the pulmonary function of the patients. In score 5 are classified patients receiving an anti-inflammatory treatment. We used the score of Aas because we have previously demonstrated that it is correlated with FEV₁, the reversibility of the airway obstruction after inhalation of salbutamol,⁵⁴ BAL EOSs,¹⁹ and with the acti-

*References 2, 6-13, 19, 23, 28, 29, 38, and 39.

vation of alveolar macrophages assessed by their capacity to release oxygen-free radicals.⁵⁵

Pulmonary function test

A pulmonary function test was performed the day before the bronchoscopy by means of a flow-volume loop done with a Pneumoscreen (E. Jaeger Laboratories, Würzburg, Germany), and normal values were analyzed according to Knudson et al.⁵⁶

Etiologic investigations of asthma

All patients had identical investigations. Allergy tests, including a battery of extracts depending on the common food and aeroallergens found in the Montpellier area,⁵⁷ were tested by skin prick tests. Total serum IgE (Phadebas PRIST and Phadiatop, Pharmacia Diagnostics AB, Uppsala, Sweden) completed the study. Serum-specific IgE was titrated by the Phadebas RAST (Pharmacia Diagnostics AB) in patients with positive skin prick tests. All patients had sinus and chest x-rays. When there was a suggestive history of aspirin intolerance, a double-blind, oral challenge was performed according to the method of Stevenson et al.⁵⁸

Fiberoptic bronchoscopy

Fiberoptic bronchoscopy was performed as previously described.²⁵ Briefly, after premedication with atropine and diazepam and local anesthesia with lidocaine, 2% applied to the upper respiratory tract, a BFTR Olympus fiberoptic bronchoscope (Olympus, Tokyo, Japan) was inserted into the trachea. The BAL was performed identically in all patients and in one of subsegmental bronchi of the middle lobe with the injection of several aliquots of sterile saline (total volume of 200 ml) reaspirated by gentle syringe suction. Each bronchoscopy was done with oxygen, and adrenaline was readily available, the patient receiving a line perfusion. A nebulization with 1 mg of salbutamol was performed after the procedure. Immediately after lavage, cytocentrifuge slides were made with an aliquot of the lavage, and the remaining BALF was centrifuged at + 4° C for 400 g for 5 minutes. The supernatant was stored at - 20° C until the assay of the mediators.

The procedure was performed in patients suffering from various stages of asthma and in some patients with severe asthma, although the current recommendations favor bronchoscopies in patients with mild asthma.^{59, 60} The safety of this study was therefore discussed in detail before starting it and approved by the ethic committee of the hospital. Three patients developed a bronchospasm during bronchoscopy that was easily reversed with 400 to 800 µg of inhaled salbutamol.

Examination of BAL cells

The BAL cytology was done on cytocentrifuged slides (cytocentrifuge Shandon Southern Products, Cheshire, England) and staining by May-Grunwald-Giemsa for EOS and by alcian blue staining for mast cells. We examined cells

on two specimens, one being done on the first lavage of 50 ml and the second being done on the remaining fluid. Since there was no significant difference between both studies except for epithelial cells, results were averaged except for epithelial cells.

Titration of ECP in BALF

ECP was titrated in BALF by a double-AB RIA (Pharmacia Diagnostics AB) with a polyclonal rabbit Ab as previously described by Venge et al.⁶¹ ECP in samples competes with a fixed amount of ¹²⁵I-labeled ECP for the binding sites of specific Abs. The ECP standards are calibrated against pure ECP prepared according to Peterson et al.⁶² The technique used was as stated on the package insert. Levels < 2 µg/L are undetectable.

Titration of tryptase in BALF

Tryptase was titrated in unconcentration BALF with a commercial assay (Pharmacia Diagnostics AB). Briefly, the assay is a solid-phase RIA based on two tryptase-specific monoclonal antibodies.^{63, 64} In the assay, the tryptase in sample reacts with antitryptase Ab bound to the wall of the test tube. Then, ¹²⁵I-labeled antitryptase is added to form a labeled complex so that tryptase in the sample reacts simultaneously with solid-phase antitryptase bound to the test tube and ¹²⁵I-labeled antitryptase forming an antitryptase-tryptase-¹²⁵I-labeled antitryptase complex. After an overnight incubation at room temperature, the tubes were washed three times, and the remaining radioactivity was determined. The intra- and interassay CVs are < 4% for samples. Levels of tryptase < 2 µg/L are undetectable.⁶⁵ The tryptase standards are calibrated against pure tryptase prepared according to Schwartz et al.⁴³

Titration of HA in BALF

HA was titrated in BALF by an RIA according to the method of Brandt et al.⁶⁶ Pure HA (Healon, Pharmacia Diagnostics AB) was used for preparation of standards and HA-Sepharose. HA-Sepharose was obtained by covalent coupling of HA to small particles of agarose (size, < 5 µm). HABPs were prepared from bovine nasal cartilage and labeled with the chloramine-T method, as previously described.⁶⁷ The test consists in incubating ¹²⁵I-labeled HABP and HA present in BALF or standard forming an ¹²⁵I-labeled HABP-HA complex. After 60 minutes of incubation, HA-Sepharose is added, and 45 minutes later, the HA-Sepharose-¹²⁵I-labeled HABP-HA formed is separated from the solution by centrifugation. The radioactivity of the gel is then determined. To assess the specificity of the test, we examined the purity of HABP, the recovery of HA in BALF, and the interaction between ECP and HA measurements. The HABP is 60% pure according to a study performed with sodium dodecylsulfate-polyacrylamide gel electrophoresis. The contamination is link protein. Two bands were found on the gel, one for HABP and another one for the link protein. The addition of HA to BALF was done, and we observed a recovery of 95.6% to 99.1%, according to the concentration of HA added. ECP does not bind to HA

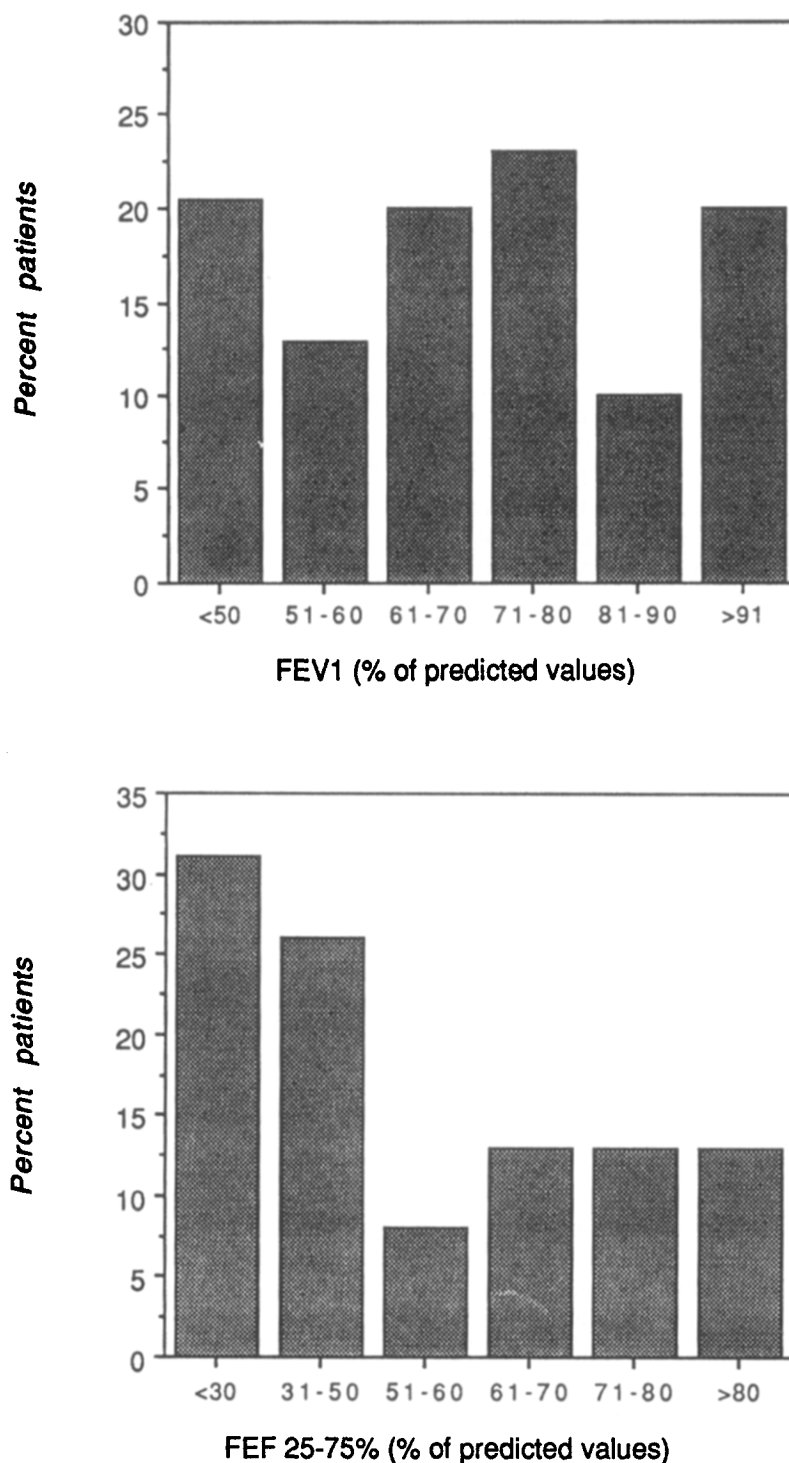


FIG. 1. Pulmonary function of the patients with asthma.

and does not interfere to the titration of HA. The intra- and interassay CVs are <10%. The detection limit is approximately 4 $\mu\text{g/L}$.

Titration of MPO in BALF

MPO was titrated by competitive radioimmunosorbent assay according to the technique of Olofsson et al.⁶⁸ previously published in detail. Briefly, anti-MPO Abs were

coupled to CNBr-activated Sephadex G-25. MPO was coupled with ^{125}I by the chloramine-T method. For the radioimmunosorbent assay, undiluted BALF was incubated with anti-MPO-Sephadex and ^{125}I -labeled MPO. After a rotating incubation of 25 hours, the anti-MPO-Sephadex particles were washed, and radioactivity of the pellet was determined. The interassay CV is <5%. The detection limit is approximately 0.5 $\mu\text{g/L}$.

Statistical analysis

Statistical analyses were done by means of nonparametric tests. Results are listed in mean $\pm$ standard deviations.

RESULTS

Characteristics of the patients

Mean ages of patients with asthma (mean $\pm$ SD, 37.8 $\pm$ 12.6 years) and normal subjects (33.6 $\pm$ 11.5 years) were similar. Seven patients had mild asthma (Aas⁵³ score of 1), 11, a score of 2; 13, a score of 3 (moderately severe asthma); and seven other patients with severe asthma (score of 4). None of the patients had a score of 5 because this implies an anti-inflammatory treatment, and none of the patients were receiving medications except inhaled β_2 -agonists (37.5% of patients). The pulmonary function of the patients was assessed by FEV₁ ranging from 35% to 130% of predicted values (mean $\pm$ SD, 72.7% $\pm$ 21.0%) and FEF_{25-75%}, ranging from 10% to 113% of predicted values (mean $\pm$ SD, 49.2% $\pm$ 28.8%) (Fig. 1). There was a significant correlation between the Aas⁵³ score and either FEV₁ ($t = 0.56$; $p < 0.0001$, Kendall's t test) or FEF_{25-75%} ($t = 0.47$; $p < 0.0001$, Kendall's t test). Twenty-two patients were allergic to perennial allergens (house dust mites and/or molds), two other patients had an aspirin intolerance, and the other patients had no demonstrable etiology (intrinsic form of asthma).

BAL cells

The recovery of BALF was significantly higher in normal subjects than in patients with asthma but did not differ in patients with asthma according to the severity of asthma. BAL EOSs were absent in the BAL of normal subjects and detectable in 19 patients with asthma, their number ranging between 1% and 15% of BAL cells. There was a significant correlation between BAL EOSs and the severity of asthma ($t = 0.31$; $p < 0.01$, Kendall t test; Table I). Polymorphonuclear cells were present in the BALF of five normal subjects and 20 patients with asthma. There was nonsignificant difference between mean neutrophil percentages or numbers in normal subjects and individuals with asthma. There was no correlation between BAL neutrophils and the severity of asthma. Metachromatic cells were always $<0.1\%$ of total BAL cells, and statistical differences were not done because of the very low number of cells. Epithelial cells were present in the first lavage fluid in 23% of subjects with asthma and were never observed in the fluid of normal individuals.

Mediator levels in BALF

ECP levels were undetectable in all normal subjects and in 14 patients with asthma. There was a significant

TABLE I. Cellular content of BALF

	Subjects with asthma	Normal subjects	
Fluid recovery (%)	37.9 $\pm$ 10.8	51.4 $\pm$ 11.0	$p < 0.01$
No. of cells ($\times 10^3$ /ml)	144.2 $\pm$ 85.1	146.0 $\pm$ 62.1	NS
Macrophages			
Percent	86.8 $\pm$ 11.8	92.5 $\pm$ 4.5	NS
Per milliliter	105.7 $\pm$ 87.5	128.2 $\pm$ 69.2	NS
Neutrophils			
Percent	2.1 $\pm$ 4.3	1.4 $\pm$ 1.9	NS
Per milliliter	2.7 $\pm$ 6.8	2.0 $\pm$ 5.2	NS
EOS			
Percent	2.1 $\pm$ 3.4	0.1 $\pm$ 0.3	$p < 0.01$
Per milliliter	2.8 $\pm$ 5.2	0.2 $\pm$ 0.6	$p < 0.01$
Lymphocytes			
Percent	8.2 $\pm$ 8.5	6.0 $\pm$ 4.1	NS
Per milliliter	9.9 $\pm$ 10.5	8.3 $\pm$ 6.2	NS
Epithelial cells			
Percent	0.92 $\pm$ 5.3	0	$p < 0.01$

NS, Not significant.

Statistical analysis by Mann-Whitney U test.

($p < 0.001$, Mann-Whitney U test) increase in mean ECP levels in patients with asthma (median $\pm$ SD, 2.55 $\pm$ 33.7 μ g/L) by comparison with normal subjects (Fig. 2). There was no correlation between ECP levels and either the age of the patients, the duration of asthma, or the etiology of asthma (allergy versus intrinsic). There was a significant correlation between ECP levels and the severity of asthma assessed by clinical score or pulmonary function (Fig. 2; Table II).

HA levels were detectable in 5/9 normal individuals and in all patients with asthma (Fig. 3). There was a significant ($p < 0.002$, Mann-Whitney U test) increase in mean HA levels in patients with asthma (median $\pm$ SD, 18.90 $\pm$ 13.4 μ g/L) by comparison with normal subjects (median $\pm$ SD, 11.96 $\pm$ 8.67 μ g/L). There was no correlation between HA levels and either the age of the patients, the duration of asthma, or the etiology of asthma (allergy versus intrinsic). There was a significant correlation between HA levels and the severity of asthma assessed by clinical score or pulmonary function (Fig. 3; Table II).

Tryptase levels were undetectable in all normal individuals and in 24 patients with asthma (Fig. 4). There was a significant ($p < 0.003$, Mann-Whitney U test) increase in tryptase levels in patients with asthma by comparison with normal subjects. There was no correlation between tryptase levels and either the age of the patients, the duration of asthma, or the etiology of asthma (allergic versus intrinsic asthma).

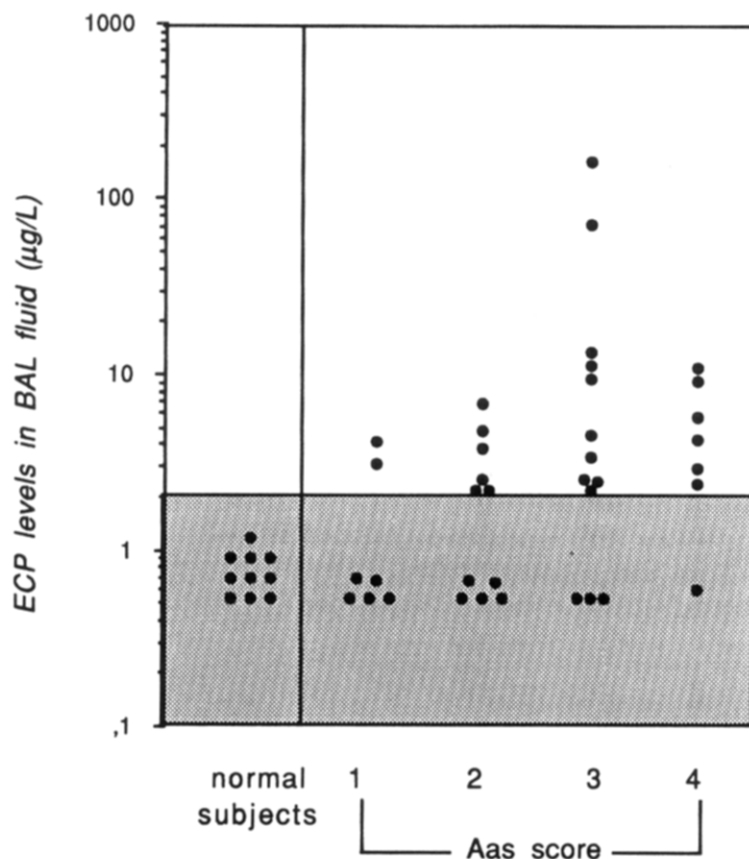


FIG. 2. ECP levels in BALF of normal subjects and patients with asthma; undetectable levels (■).

TABLE II. Correlations observed between results

	Test used	ECP	Tryptase	HA	MPO
Age of the patients	Spearman's	NS	NS	NS	NS
Duration of asthma	Spearman's	NS	NS	NS	NS
Severity of asthma					
Aas score	Kendall's	$t = 0.28; p < 0.01$	NS	$t = 0.33; p < 0.005$	NS
FEV ₁	Spearman's	$\rho = 0.40; p < 0.02$	NS	$\rho = 0.56; p < 0.001$	NS
FEF ₂₅₋₇₅	Spearman's	$\rho = 0.44; p < 0.01$	NS	$\rho = 0.54; p < 0.002$	NS

NS, Not significant.

There was no significant correlation between tryptase levels and the severity of asthma assessed by clinical score or pulmonary function (Fig. 4; Table II).

MPO levels were undetectable in 6/9 normal individuals and in 11 individuals with asthma (Fig. 5). Only seven patients with asthma had MPO levels higher than levels observed in normal individuals. Three or the four patients with MPO levels >30 µg/L were former smokers. There was no significant (Mann-Whitney U test) increase in mean MPO levels in patients with asthma (median $\pm$ SD, 4.0 ± 30.75 µg/L) by comparison with normal subjects (me-

dian $\pm$ SD, 3.50 ± 4.64 µg/L). There was no correlation between MPO levels and the severity of asthma assessed by clinical score or pulmonary function (Fig. 5; Table II).

Correlations between BAL parameters

Correlations between mediators revealed a highly significant correlation between ECP and HA levels (Fig. 6; $\rho = 0.62; p < 0.001$, Spearman's rank test). There was no significant correlation between BAL EOSs and ECP nor between BAL neutrophils and MPO levels.

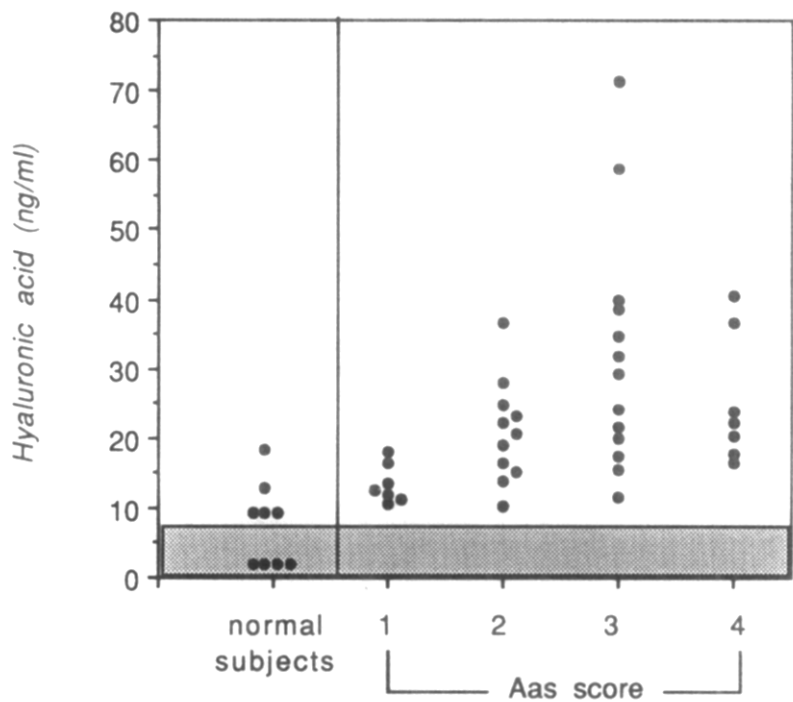


FIG. 3. HA acid levels in BALF of normal subjects and patients with asthma; undetectable level (■).

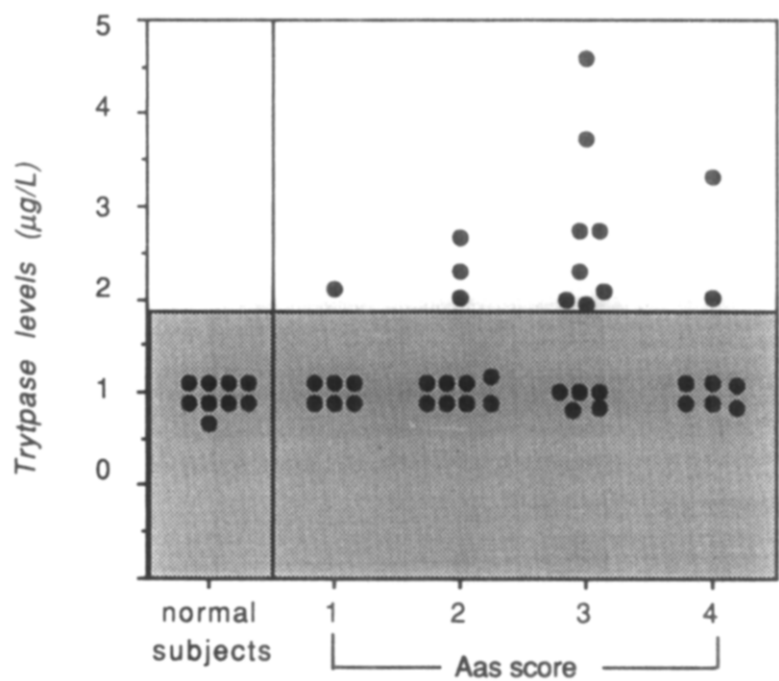


FIG. 4. Tryptase levels in BALF of normal subjects and patients with asthma; undetectable level (■).

DISCUSSION

This study examined four inflammatory mediators released in BALF from patients with asthma of variable severity (FEV₁ ranging from 35% to 130% of

predicted values) and demonstrated that all mediators but MPO were increased in patients with asthma by comparison with normal individuals. There was a significant correlation between the severity of asthma

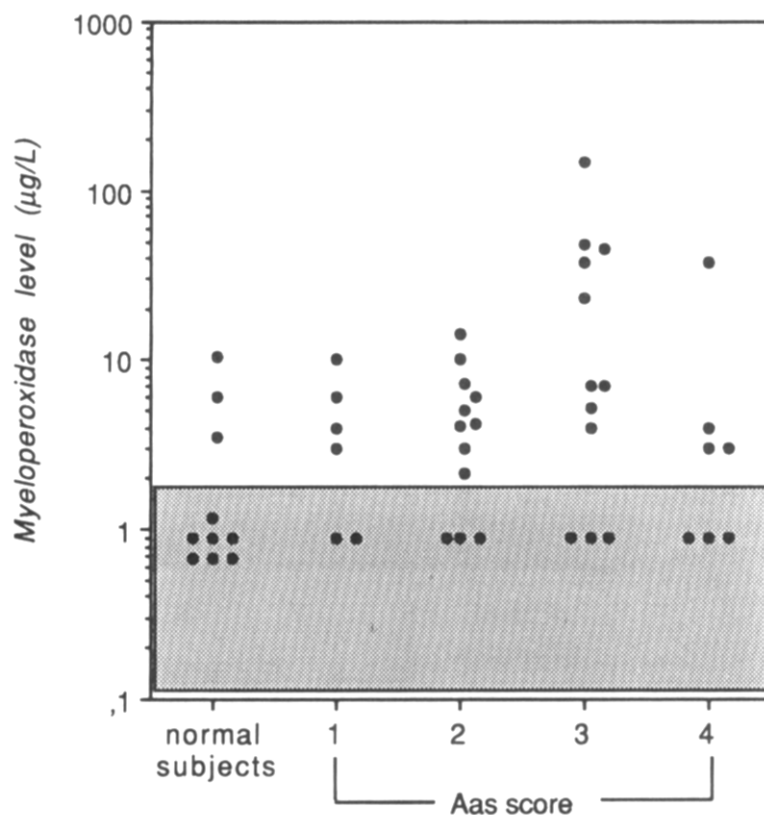


FIG. 5. MPO levels in BALF of normal subjects and patients with asthma; undetectable levels (■).

assessed by clinical score or pulmonary function and EOS-derived mediators (ECP) and HA (assessing mainly fibroblast activation), but no such correlation was found for the mast cell-derived mediator (tryptase) or the neutrophil-derived mediator (MPO). The BALF levels of ECP and HA, as well as of ECP and tryptase levels, were significantly correlated, suggesting common or sequential activation mechanisms. These markers may have a value in the study of bronchial inflammation in asthma.

Airway inflammation can be studied by different methods. Bronchial biopsy specimens directly appreciate inflammatory cells and airway damage, but biopsies are performed on large airways, cannot give quantitative results, and do not appreciate the possible heterogeneity of airway lesions. Thus, in this study, we used BAL, since it indirectly appreciates airway inflammation, provides information on a wider part of the airways, and is quantitative. However, this method suffers from some defects, since it examines ill-defined sites of the airways, recovers fluid from alveoli and bronchi, and some BALF mediators may be degraded.

The assessment of the clinical score of asthma is difficult, and although there appears to be no perfect

assessment, we decided to use the score of Aas⁵³ since we had previously observed a correlation with the pulmonary function,⁵⁴ EOS counts in peripheral blood or BALF,¹⁹ and the activation of alveolar macrophages.⁵⁵ We measured the nonspecific bronchial hyperreactivity in a few patients only, since bronchial challenge is recommended with nonspecific agents when FEV₁ is >70% of predicted values.⁵² Bronchoscopy is an invasive procedure commonly accepted in patients with mild asthma,^{59, 60} but we performed the procedure in 12 patients with FEV₁ <60% of predicted values. The study was done after having discussed the feasibility of bronchoscopy in such patients. None of the patients of the present study had a severe asthmatic attack during the procedure.

The results of a specific marker in BALF may be expressed as its concentration in original "neat" BALF as micrograms per milliliter of fluid, calculated as a percent of total protein, or as a ratio with albumin⁶⁹ or urea.⁷⁰ Although the BALF recovery was different in patients with asthma and in normal subjects, we expressed the results in micrograms per milliliter of BALF since (1) the instilled volume was always the same and calculations of percentages or ratios did not therefore appear necessary,⁷¹ and (2) the choice of

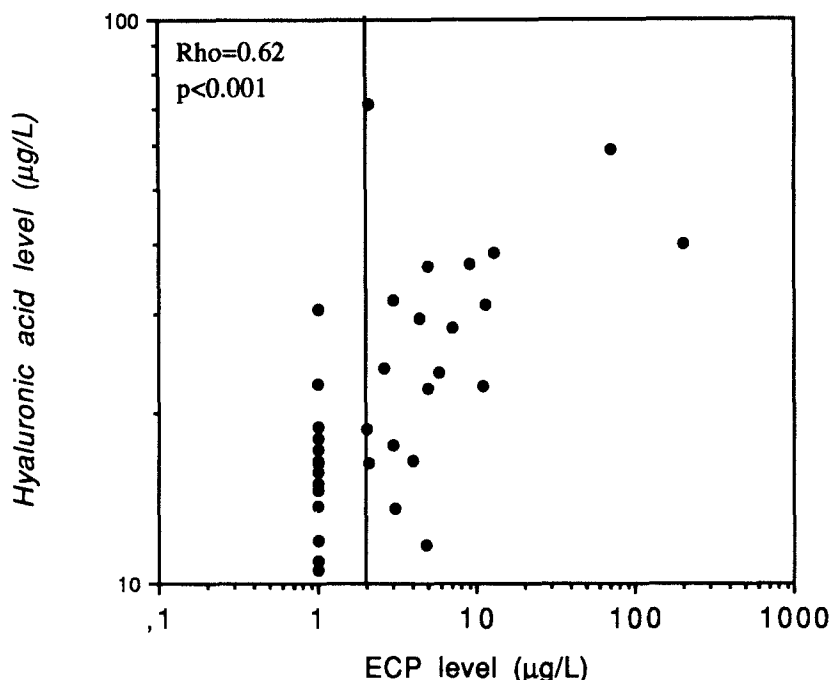


FIG. 6. Correlation between ECP and HA levels in BALFs of patients with asthma.

albumin or urea to express the results is not yet completely validated.^{70, 71} We selected the EOS-specific protein, ECP, rather than other EOS granule proteins, such as MBP, since (1) MBP is less specific for the EOS,⁷² (2) ECP appears to be more cytotoxic than MBP and is considered by far to be the most potent tissue-damaging protein of the EOSs,⁷³ and (3) the titration of ECP by RIA is highly reproducible and commercially available. Tryptase is a mast cell-specific marker found in both types of mast cells and is released during allergen challenge.^{36, 41-43} It was selected as a mast cell-derived mediator because it appears to be more stable than histamine or PGD₂, and its titration by RIA is highly reproducible and commercially available. Usually BALF should be concentrated to obtain detectable tryptase levels, but we preferred to use unconcentrated BALF since more than one third of the subjects with asthma had detectable levels, and the concentration of BALF is difficult to standardize, leading to some variability in the results.⁷¹ HA is the largest glycosaminoglycan ubiquitously distributed in tissues and body fluids. Although it is produced by mast cells and leaking lymph, the largest amounts of HA in BALF are likely to be synthesized by activated fibroblasts,^{44, 74} and its synthesis was correlated with fibroblast proliferation⁷⁵ as well as its release during lung fibrotic diseases.⁴⁶⁻⁴⁸ MPO titrated by RIA is a specific marker of polymorphonuclear phagocytes.

One of the originalities of this article is in its study

of many untreated subjects with asthma and some patients with severe asthma. No patient had received any anti-inflammatory treatment that might affect the results of the study. β_2 -agonists were the only treatment received by 37.5% of the patients. It has been suggested that these drugs can affect peripheral blood EOSs^{76, 77} in normal individuals and to a lesser extent in patients with asthma,⁷⁸ but there are studies demonstrating no in vitro effect.⁷⁹ The effect of β_2 -agonists on mast cells is more profound.⁸⁰ In vitro studies have demonstrated that these drugs decrease greatly histamine release from human chopped lung mast cells.⁸¹ These effects were also found in in vivo animal studies.⁸² During allergen bronchial challenge, β_2 -agonists can reduce or even abrogate the early phase reaction^{83, 84} as well as the release of histamine and chemotactic mediators.⁸⁴ In contrast, the effects of β_2 -agonists on the release of tryptase are unknown. In the present study, none of the patients were receiving oral drug or long-acting β_2 -agonist, and inhaled β_2 -agonists had been stopped for 8 hours before the BAL. Thus, the use of β_2 -agonists does not appear to alter greatly the results of this study, and it would have been unethical to withdraw these drugs for more than 8 hours in patients with severe asthma currently receiving this treatment.

BALF ECP levels are increased in patients with asthma and correlated with the severity of asthma. This study confirms a previous study¹⁹ in which patients with less severe asthma were tested. Thus, there

is a link between the severity of asthma and the degree of EOS degranulation. In the former study, ECP-immunohistochemistry study of bronchial biopsy specimens suggested a role for ECP in the shedding of the epithelium. These two studies combined with other postmortem pathologic examinations¹² indicate that EOSs are major deleterious cells of the airway inflammation in subjects with asthma. In the patients with the most severe asthma (Aas⁵³ score of 4), mean levels of ECP were lower than in patients having Aas⁵³ score of 3. This result might be explained by the low number of patients with severe asthma as well as by the natural course of chronic inflammation during which the release of inflammatory mediators decreases in the latest stages and leaves a place to repair process. However, it must be pointed out that the percentage of patients with presence of ECP in BALF increases with the severity of asthma, although mean values decreased.

Tryptase was never found at a detectable level in BALF of normal subjects. In contrast, it was present in 36.8% of unconcentrated BALF of patients with asthma, indicating mast cell activation in asthma as already observed in pathologic studies^{14, 17} and suggested from BALF analysis^{30, 36} after concentration of the fluid. However, there was no correlation between tryptase levels and the severity of asthma, possibly because we used unconcentrated BALF, and many patients had undetectable levels. Tryptase was released similarly in patients with allergic and intrinsic asthma, demonstrating that mast cells are not only triggered by allergen in asthma.

HA was present in BALF of 5/9 normal subjects and BALF of all patients with asthma. Mean levels were significantly greater in patients with asthma than in control subjects, as previously observed.³⁷ Moreover, HA levels were significantly correlated with the severity of asthma, suggesting a fibroblast activation in asthmatic airways and/or a lymph leakage in the airways. This finding is in agreement with the study of Roche et al.¹⁸ who found a subepithelial fibrosis and suggests that this may be due to myofibroblast activation. The findings of Roche et al.¹⁸ and Bousquet et al.²⁰ indicate that pathologic abnormalities of asthmatic airways include a destructive component that may be partly mediated by the activation of fibroblasts, leading to an abnormal healing process characterized by the accumulation of collagen beneath the basement membrane.

MPO levels were undetectable in 6/9 normal individuals and in 11 subjects with asthma. There was no significant increase in mean MPO levels in patients with asthma by comparison with normal subjects, and 3/4 patients with MPO levels >30 µg/L were former smokers. These findings confirm pathologic studies

demonstrating that neutrophils are not increased in bronchi of subjects with asthma^{85, 86} and suggests that neutrophil activation is not a key feature of chronic asthma except possibly in smokers with asthma.

In the present study, there is a highly significant correlation between ECP and HA levels in BALF, suggesting a common or sequential activation of EOSs and fibroblasts. It is not known whether EOSs, fibroblasts, and mast cells are coactivated by themselves or by other cell types or, alternatively, whether there is a first cell that is activated and in turn stimulates the other cells. It has, however, been observed that EOSs and metachromatic cells can be activated by cytokines,^{86, 87} some of these cells released by fibroblasts,⁸⁸ and that fibroblasts are activated by EOSs.⁸⁹ EOSs can also activate mast cells since MBP induces degranulation,⁹⁰ and mast cells may stimulate fibroblasts since histamine inhalation increases HA levels in BALF. It is therefore possible that inflammation mediates fibroblast activation, and this concept is of importance since it suggests that inflammation may induce airway destruction as well as it suggests some importance in the effector cell function of fibroblasts.

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