

Protein content in bronchoalveolar lavage fluid of patients with asthma and control subjects

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Background: Secretory component (SC), Clara cell protein (CC10) and to some extent, IgM are proteins locally synthesized in airways. Albumin, α 2-macroglobulin and α 1-antitrypsin are mainly plasma proteins. In patients with asthma, blood proteins may occur in greater amounts in bronchoalveolar lavage fluid (BALF) than in control subjects because of plasma extravasation. These proteins were measured in BALF to define markers of local synthesis and plasma exudation.

Methods: Twenty-four patients with asthma (mean age, 40 ± 3.1 years) and 24 control subjects (mean age, 29 ± 11 years) were tested. Five aliquots of saline solution (50 ml) were instilled, and the recovered BALF was stored. Clara cell protein was measured by a sensitive immunoassay technique based on the agglutination of latex particles. SC, immunoglobulins, α 2-macroglobulin, α 1-antitrypsin, and albumin were measured by an immunoradiometric assay. Protein concentrations were normalized to albumin.

Results: In BALF from patients with asthma there was a significant increase in α 2-macroglobulin and IgM and a significant decrease of SC, α 1-antitrypsin, and Clara cell protein compared with control subjects. Also, the ratio to albumin was significantly increased for α 2-macroglobulin and IgM and decreased for SC. There was no correlation between the severity of asthma and the amount of proteins.

Conclusions: BALF protein content is significantly different in patients with asthma and control subjects, for proteins produced locally and derived from plasma. (*J ALLERGY CLIN IMMUNOL* 1995;95:60-8.)

Key words: Bronchoalveolar lavage, asthma, proteins, secretory component, Clara protein (CC10)

Asthma is a chronic inflammatory disease involving plasma extravasation which leads to exudation of proteins.^{1,2} Increased levels of α 2-macroglobulin (α 2-M), IgM, and albumin levels measured in bronchoalveolar lavage fluid (BALF) from patients with asthma³⁻⁵ are likely to reflect this plasma extravasation. However, IgM⁵ and to a lesser extent α 2-M⁶ may also be produced locally.

On the other hand, some proteins are synthe-

Abbreviations used

α 1-AT:	α 1-antitrypsin
α 2-M:	α 2-macroglobulin
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
CC10:	Clara cell protein (10 kd)
FEV ₁ :	Forced expiratory volume in 1 second
SC:	Secretory component

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tized locally by nonciliated bronchial cells, and decreased contents in BALF may reflect an abnormal function of or damage to the epithelial cells.^{7,8} Secretory component (SC) is a glycoprotein synthesized by serous epithelial cells, which line the respiratory tract,^{9,10} making possible the active transport of dimeric IgA through epithelial cells into the epithelial lining fluid¹¹ where it is present

as free SC or IgA-bound SC.¹² CC10 is a 10 kd protein, which has been identified in Clara cells, nonciliated cells localized in the bronchiolar epithelium^{13,14} and in nonciliated columnar cells of large and small bronchi, as well as in bronchioles.¹⁵ CC10 can be measured in human BALF.^{8,13,16} Previous studies have reported decreased concentration of SC⁷ and CC10⁸ in BALF of smokers and decreased CC10 levels in BALF from patients with chronic bronchitis,⁸ which may suggest an impairment of the epithelial cell secretory function.

Various proteins were studied in BALF from 24 patients with asthma and 24 healthy subjects (control subjects) to compare plasma-derived and locally synthesized proteins. The levels of these proteins were correlated to the severity of asthma as assessed by the clinical score of Aas,¹⁷ forced expiratory volume in 1 second (FEV₁), and histamine levels in BALF.

METHODS

Patients

We studied 24 patients with asthma (age range, 18 to 69 years; mean age $\pm$ SD, 40 $\pm$ 3.1 years). Asthma was defined as previously described¹⁸ according to the criteria of the American Thoracic Society,¹⁹ and all patients had a reversible airway obstruction characterized by a 15% increase in FEV₁ values after the inhalation of 200 μ g of albuterol. The clinical severity of asthma was assessed according to the score of Aas,¹⁷ which grades the different forms of chronic asthma on the basis of the events that took place during the previous year and combines symptoms (number and duration of asthmatic episodes, total duration of symptoms, presence or absence of symptom-free intervals between attacks) and the need for medication. The score ranges from very mild (1) to very severe (5) forms of asthma but does not take into account the pulmonary function of the patients. In addition, the score of Aas¹⁷ is correlated with FEV₁,¹⁸ eosinophilic inflammation,¹⁸ the activation of alveolar macrophages (as assessed by their capacity to release oxygen-free radicals), and the expression of surface receptors on epithelial cells in asthma.²⁰ None of the patients were current smokers, and none had smoked within the previous 2 years. Patients were excluded from the study if they had taken systemic corticosteroids in any form during the previous 2 months; if they had used inhaled corticosteroids during the previous month; or if they had taken nedocromil sodium, cromolyn sodium, or ketotifen during the previous week or theophylline during the 48 hours before the test. Treatment with β_2 -agonists was withheld only for 8 hours before the trial. Allergy was defined with the criteria described in a previous article.¹⁸

We also studied 24 normal nonsmokers (18 to 65 years; mean $\pm$ SD, 29 $\pm$ 11 years) as a control group. Their pulmonary function was in the normal range. They had no allergic diseases and had never had asthma.

The study was approved by the Ethics Committee of the University of Montpellier, and the patients gave informed consent.

Fiberoptic bronchoscopy

Fiberoptic bronchoscopy was performed as previously described.^{18,21,22} The bronchoalveolar lavage (BAL) was carried out in one of subsegmental bronchi of the middle lobe by injecting 5 aliquots of saline solution (50 ml) at room temperature, reaspirated by gentle syringe suction. Immediately after lavage, mucus was removed from the fluid by filtration through gauze.

Examination of BAL cells

After recovery, BALF was strained through a monolayer of surgical gauze to remove mucus. After mixing, an aliquot of 5 ml of each sample was used to obtain the total cell count with a hemocytometer. Cells were examined by an investigator who was unaware of the identity of the subject group. Cell differential counts were made, after cytocentrifugation (Cytospin; Shandon Scientific Ltd., Cheshire, U.K.) and staining with May-Grünwald-Giemsa stain, by counting 200 cells on each slide. Macrophages, lymphocytes, eosinophils, neutrophils, and epithelial cells (including ciliated and goblet cells) were counted, and results were given as percentages. Unconcentrated supernatant was frozen at -20°C before proteins were measured.

Measurement of proteins in BALF

The CC10 content was determined with a sensitive immunoassay method based on the agglutination of latex particles,^{23,24} which has been recently described in detail.²³ This assay has been used and validated in BALF.⁸

The total SC content of the BALF was determined by immunoradiometric assay.²⁵⁻²⁷ This assay²⁷ measures all forms of SC by reference to an 11S p-IgA-SC standard purified from human milk.²⁶

The other proteins (albumin, α_1 -antitrypsin [α_1 -AT], α_2 -M, IgG, IgM, and IgA) were also quantitated by immunoradiometric assay.¹²

For comparison purposes, protein concentrations were normalized to albumin.

Measurement of histamine in BALF

Histamine was measured by means of a radioimmunoassay with a monoclonal antibody raised against acetylated histamine (Immunotech, Luminy, France).²⁸

Statistical analysis

Statistical analyses were performed with a Macintosh Computer (Apple Computer, Inc., Cupertino, Calif.) with Statview II software (Statview, Inc., Berkeley, Calif.). Comparisons were made by two-tailed nonparametric tests. Correlation coefficients were calculated with the Spearman's rank test. Results are expressed as means $\pm$ SEM. Bonferroni's correction was used when multiple data were analyzed.

TABLE I. Characteristics of asthmatic patients

Aas score	n	Age (yr)*	Sex		FEV ₁ (%)*
			M	F	
1	5	24 ± 2.5	2	3	95.0 ± 2.5
2	9	35 ± 4.7	5	4	90.3 ± 7.2
3	7	50 ± 5.0	3	4	66.6 ± 7.5
4	3	48 ± 1.2	2	1	53.8 ± 1.2

*Values expressed as means ± SEM.

RESULTS

Characteristics of the patients and tolerance to BAL

Details of the patients who participated in the study are shown in Table I. Five patients had an Aas score of 1 (mild asthma), 9 had a score of 2 (moderate asthma), 7 had a score of 3 (moderately severe asthma), and 3 had a score of 4 (severe asthma). No patient had a score of 5, because this level of severity requires corticosteroid treatment, and the patients received no medications except inhaled β_2 -agonists. FEV₁ values ranged from 42% to 122% of predicted values (mean ± SEM, 84.0% ± 4.8%). Twelve patients were allergic. Tolerance to the BAL procedure was excellent: there was no severe attack during endoscopy, and only one subject experienced mild exacerbation of asthma, which did not require the cessation of the procedure and was resolved after nebulization with 1 mg of salbutamol.

BALF recovery

The BALF recovery was significantly decreased ($p = 0.02$, Mann-Whitney U test) in patients with asthma (119.8 ± 3.6 ml; $47.9\% \pm 1.45\%$) in comparison with the control subjects (150.0 ± 3.0 ml; $60.0\% \pm 1.2\%$). The volume of BALF recovered was not significantly correlated with the FEV₁ value (Spearman's rank correlation).

Cell content of BALF

The cell content of BALF is presented in Table II. In patients with asthma eosinophil numbers were significantly ($p < 0.005$, Mann-Whitney U test) increased.

Protein content of BALF

The protein content of the BALF is presented in Table III and Figs. 1 and 2. A significant increase of α_2 -M and IgM levels was observed in BALF from patients with asthma as compared with control subjects. In contrast, α_1 -AT, CC10, and SC levels were significantly decreased in BALF from

patients with asthma. When the results were expressed as a ratio to albumin, significant differences between the asthmatic and control groups were observed for α_2 -M, IgM, and SC. No correlation was found between the levels of these proteins and BALF recovery.

Histamine content of BALF

The histamine concentration and ratio to albumin in BALF from patients with asthma are presented in Table III. A significant increase of histamine levels was observed in BALF from patients with asthma (Mann-Whitney U test, $p < 0.001$). However, there was no correlation between the levels of histamine and BALF recovery.

A significant correlation was observed between the BALF contents of histamine and albumin, α_2 -M, or IgM (Fig. 3). The correlation between the BALF contents of histamine and IgM was no longer significant when Bonferroni's correction was applied. There was no significant correlation between histamine levels and proteins locally synthesized.

DISCUSSION

This study confirms that two plasma-derived proteins (α_2 -M and IgM) are increased in BALF from patients with asthma. However, albumin concentrations showed no such increase. Moreover, a significant correlation was observed between histamine and proteins derived from plasma, albumin, and α_2 -M. On the other hand, two locally produced proteins, SC and CC10, were decreased in BALF from patients with asthma, suggesting a possible abnormal behavior of epithelial cells.

In BALF the presence of proteins such as albumin, α_2 -M, and to a lesser extent IgM represents indirect evidence of increased vascular permeability.⁴ In this study we decided to measure the BALF concentrations of plasma-derived proteins with different molecular weights (albumin and α_2 -M)^{4, 5, 29} and the levels of histamine, a mediator that increases vascular endothelial permeability.³⁰ Further, we studied some locally synthesized proteins (SC protein and CC10)^{8, 10, 26} in order to assess differences in local synthesis between patients with asthma and control subjects. This study is original because it is the first in which several locally synthesized and plasma-derived proteins are studied and compared with histamine, which enhances vascular permeability.

The accurate quantitation of soluble components in BALF requires that the variable dilution of the epithelial lining fluid during lavage be taken

TABLE II. Comparison between BALF differential cell counts in patients with asthma and control subjects

	Patients with asthma	Control subjects
BALF recovery (%)	47.9 ± 1.45*	60.0 ± 1.2
Number of cells (1000 cells/ml)	146.8 ± 27.5	178.3 ± 24.4
Macrophages (%)	74.6 ± 3.74	83.1 ± 3.44
Lymphocytes (%)	8.80 ± 1.46	10.4 ± 2.1
Eosinophils (%)	3.08 ± 1.17*	0.5 ± 0.3
Neutrophils (%)	5.58 ± 1.64	2.33 ± 0.96
Epithelial cells (%)	4.75 ± 1.25	3.6 ± 2.25

Results are expressed as means ± SEM.

* $p < 0.05$ (Mann-Whitney U test) for difference between patients with asthma and control subjects.

TABLE III. Protein and histamine content of BALF (Mean ± SEM)

	Patients with asthma	Control subjects	<i>p</i> Value
CC10 (μg/ml)	2.13 ± 0.29	3.18 ± 0.33	0.015
Albumin (μg/ml)	18.0 ± 1.5	23.2 ± 2.07	NS
α1-AT (μg/ml)	0.28 ± 0.06	0.56 ± 0.11	0.020
α2-M (μg/ml)	0.15 ± 0.03	0.09 ± 0.02	0.0083
IgG (μg/ml)	6.5 ± 0.90	8.70 ± 1.74	NS
IgM (μg/ml)	0.16 ± 0.03	0.09 ± 0.02	0.035
IgA (μg/ml)	1.80 ± 0.20	2.13 ± 0.24	NS
SC (μg/ml)	2.10 ± 0.20	5.50 ± 0.81	0.001
Histamine (pg/ml)	878.37 ± 125.7	80.04 ± 20.8	0.0001
CC10/Alb (pg/ml)	0.140 ± 0.031	0.187 ± 0.037	NS
α1-AT/Alb (pg/ml)	0.015 ± 0.003	0.024 ± 0.003	NS
α2-M/Alb (pg/ml)	0.009 ± 0.002	0.003 ± 0.001	0.0003
IgG/Alb (pg/ml)	0.341 ± 0.036	0.347 ± 0.041	NS
IgM/Alb (pg/ml)	0.010 ± 0.002	0.004 ± 0.001	0.0015
IgA/Alb (pg/ml)	0.106 ± 0.013	0.097 ± 0.009	NS
SC/Alb (pg/ml)	0.127 ± 0.014	0.265 ± 0.039	0.0106
Histamine/Alb (pg/ml)	40.95 ± 9.04	3.75 ± 1.18	0.0001

Statistical analysis was done by Mann-Whitney U test.

Alb, Albumin; NS, not significant.

into consideration. In an attempt to control this problem, it has been proposed that the data be expressed as a ratio of the concentration of a given soluble component to the concentration of total protein or albumin.³¹⁻³⁶ However, the use of albumin as a reference standard has limitations because some diseases, including asthma, are often associated with increased vascular permeability and the volume of the instillate.^{4, 31, 32, 37} Among the other possible dilution markers, urea has been suggested³⁸; however, this is not an ideal choice because urea concentrations also depend on vascular permeability.³⁹⁻⁴¹ At the moment, there is no completely reliable method to correct for variable dilution,⁴² and we therefore chose to use the ratio to albumin. Another important factor to be con-

sidered is the percentage recovery of BALF, because it has been suggested that decreased BALF recovery is associated with an underestimation of the concentration of BAL-soluble components.⁴³ In this study BALF recovery was significantly different in both groups, and hence protein levels may have been underestimated in patients with asthma. However, no correlation was observed between BALF recovery and the levels of proteins or histamine recovered. Indeed, the ratio of the soluble components to albumin is likely to correct for variable dilution of BALF and may represent a more accurate expression of the data. However, because there is a significant correlation between albumin and histamine levels, the expression of the data as a ratio of albumin may not be com-

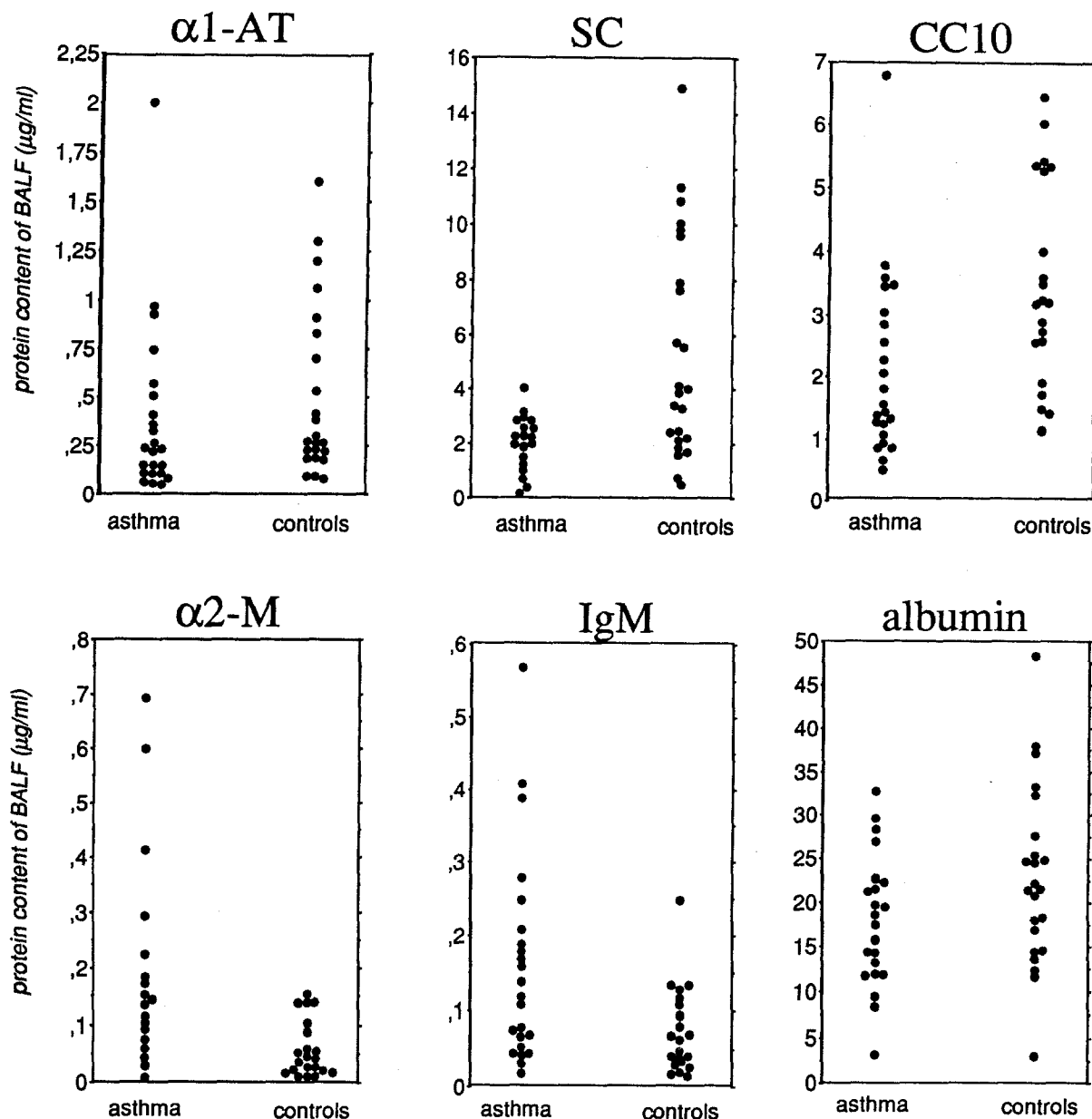


FIG. 1. BALF protein content in patients with asthma and control subjects.

pletely satisfactory. These are the reasons for presenting our protein data both as absolute values and as their ratio to albumin.

Moreover, we observed no significant correlation between BALF recovery and FEV_1 . This suggests that the relationship between bronchial obstruction and BALF recovery appears more complex than might be expected.

In this study the BALF albumin content was not found to be significantly increased in patients with asthma. This result may appear surprising, but BALF albumin content was not found to be increased in some studies,^{29, 44, 45} whereas other au-

thors reported an increase.⁴ These differences may be associated with the heterogeneity of the disease in some patients with asthma because an increase in albumin BALF content was reported in asthmatic subjects after challenge but not under basal conditions.^{44, 46} The increase in $\alpha 2$ -M BALF content observed in this study was previously observed in BALF from patients with asthma,⁴ suggesting an increased vascular permeability. The differences between albumin and $\alpha 2$ -M levels may be due to the increased passive diffusion of $\alpha 2$ -M, or active transendothelial and epithelial transport, or local production.³ In this study increased histamine lev-

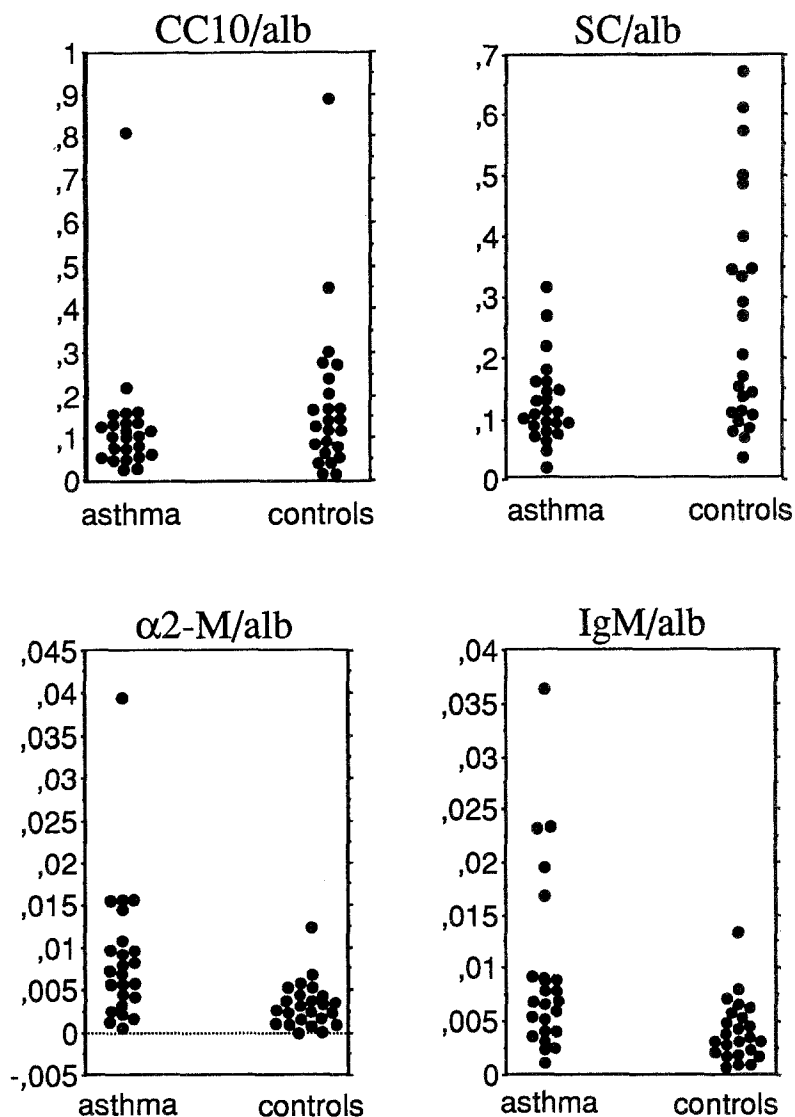


FIG. 2. BALF protein content in patients with asthma and control subjects (ratio to albumin).

els were observed in BALF from patients with asthma, as previously demonstrated.⁴⁷⁻⁵¹ Moreover there was a significant correlation between histamine and α 2-M or albumin, but the correlation was closer for α 2-M than for albumin. The increased vascular permeability induced by histamine³⁰ is likely to explain these correlations. Alternatively, because histamine is present in plasma, the increased levels in BALF may reflect a passage of this mediator from blood vessels to the airway lumen.

IgM levels in BALF from patients with asthma were significantly increased compared with those of control subjects. The IgM content in BALF may be related to transudation from plasma, and as previously suggested, to local production by sub-

mucosal plasma cells.⁵ In BALF of patients with asthma, α 1-AT was lower than in control subjects, as previously reported,⁴⁴ whereas the ratio to albumin was not significantly different between the two groups, suggesting a variable dilution in patients with asthma and control subjects.

SC and SC ratio to albumin were significantly decreased in patients with asthma. SC is a glycoprotein synthesized by serous epithelial cells lining the respiratory tract^{9,10} that can be measured in BALF as free SC,^{7,12,34} IgA-bound SC,¹² or total SC.⁸ It has been reported that free SC was decreased in BALF from smokers compared with control subjects, suggesting a possible dysfunction of the epithelial cells.⁷ In contrast, total SC was not reduced in BALF from both smokers and patients

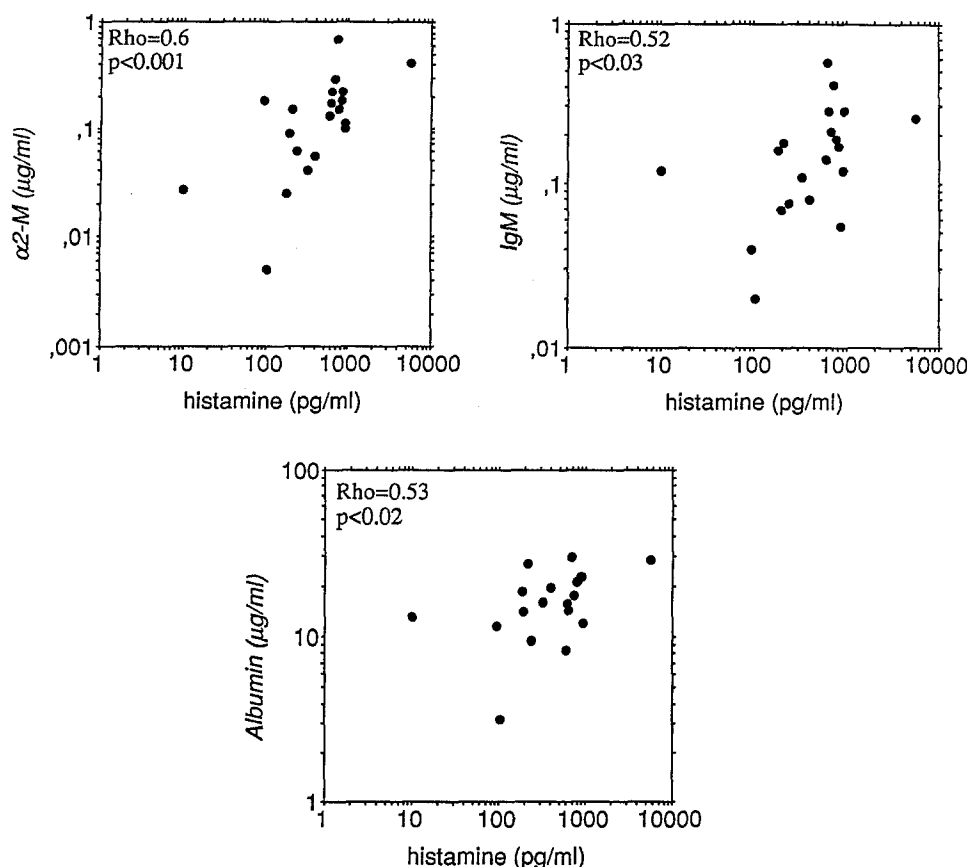


FIG. 3. Correlation between histamine and protein BALF content (by Spearman's rank test).

with chronic bronchitis.⁸ In patients with asthma, an increased IgA-bound SC content was observed in BALF, as compared with control subjects.⁵² Nevertheless, the measurement of total SC is unlikely to be affected by the synthesis of secretory IgA.

CC10 is a 10 kd protein, which has been identified in human Clara cells^{13, 14} and BALF.^{13, 16} It is synthesized by human Clara cells,^{13, 14} nonciliated cells localized in bronchiolar epithelium, whereas CC10 has also been recently observed in nonciliated columnar cells in the large and small bronchi, as well as in bronchioles.¹⁵ Decreased levels of SC and CC10 in the BALF of patients with asthma suggest that the turnover of epithelial cell-derived proteins may be altered in asthma.

We were unable to find any significant correlation between any BALF protein and the severity of asthma as assessed by FEV₁ values or the clinical scoring system of Aas.¹⁷ The lack of correlation with the severity of asthma is not surprising and could easily be related to the complexity of the disease.

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