Increased Levels of Elastase and α_1-Antitrypsin in Sputum of Asthmatic Patients

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Asthma and chronic bronchitis are inflammatory diseases associated with remodeling of the extracellular matrix (ECM). Elastin, a major component of the ECM in the airways, has been previously found to be disrupted in asthma and chronic bronchitis. This study was aimed at evaluating whether elastin disruption might be associated with an imbalance between elastase (active and total) and α_1-proteinase inhibitor (α_1-PI), the main inhibitor of elastase. We measured elastase and α_1-PI in induced sputum obtained from 16 control subjects, 10 healthy smokers, 19 asthmatic patients, and 10 chronic bronchitis patients. We also assessed the possible origin of elastase, evaluating its levels in sputum with reference to differential cell counts. We found that in induced sputum obtained from asthmatic and chronic bronchitis patients, the levels of both total and active elastase were significantly increased as compared with those of control subjects and healthy smokers and were significantly correlated with the percentage of neutrophils. In addition, in asthma and chronic bronchitis patients, the levels of active and total elastase were inversely correlated with the degree of airway obstruction as assessed from FEV_1 values. This study shows that airway inflammation in asthma and chronic bronchitis is associated with high levels of active elastase, which may play a role in the pathogenesis of airway remodeling. Vignola AM, Bonanno A, Mirabella A, Riccobono L, Mirabella F, Profita M, Bellia V, Bousquet J, Bonsignore G. Increased levels of elastase and α_1-antitrypsin in sputum of asthmatic patients.

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The extracellular matrix (ECM) of the lung is a dynamic structure, in which an equilibrium between synthesis and degradation of components is required for the maintenance of homeostasis. Tissue remodeling requires the controlled degradation of ECM molecules by proteases and protease inhibitors. Many cells participating in airway inflammation may secrete enzymes capable of degrading ECM proteins, including elastin (1, 2). The major source of elastase in human lung is represented by the neutrophil (3); another potential source is the macrophage (1). The main inhibitor of elastase is α_1-antitrypsin (α_1-AT) (4). Because α_1-AT can also inhibit other proteinases, it is often referred to as α_1-proteinase inhibitor (α_1-PI). α_1-PI is produced by the liver and diffuses from the blood to the lungs, from which it can be recovered in bronchial secretions.

Proteolytic enzymes have the potential to destroy lung structures, provided that their activity exceeds the capacity of their natural inhibitors. Neutrophil elastase is regularly present in secretions from patients affected by chronic obstructive pulmonary disease (COPD) (5): an imbalance between elastase and α_1-PI is assumed as a prominent pathogenetic mechanism of emphysema (6); fibrosis of the small airways is an additional aspect of ECM remodeling in this disease (7).

Asthma is a chronic inflammatory disease of the airways (8) associated with ECM remodeling, including subepithelial fibrosis (9); in a previous study, we found evidence of degradation of elastin as assessed immunohistochemically in bronchial biopsies obtained from the large airways of asthmatic subjects (10). The present study was directed at evaluating whether this histologic evidence might be related to an imbalance between elastase and α_1-PI measured in sputum. The latter was induced by inhalation of hypertonic saline by 16 control subjects, 10 healthy smokers, 19 asthmatic subjects, and 10 subjects affected by chronic bronchitis. We also assessed the possible origin of elastase by referring its levels in sputum to differential cell counts.

METHODS

Patients

The study was done on four groups of subjects. The study groups included 16 control subjects, 10 healthy smokers, 19 patients with asthma, 10 patients with bronchitis and/or COPD, respectively. Asthmatic subjects were 19 to 67 yr of age (median and percentiles: 45 and 24 to 64 yr, respectively). Asthma was diagnosed on the basis of criteria previously described in detail (11). None of the subjects was a current or previous smoker. Patients were excluded from the study if they had undergone a severe exacerbation of asthma requiring hospitaliza-

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tion during the month preceding the study. Inhaled corticosteroids or oral corticosteroids had been withdrawn for at least 2 mo prior to the commencement of the study, and the use of nedocromil sodium or cromoglycate had been stopped for at least 2 wk and theophylline for 48 h prior to the study.

Ten patients with chronic bronchitis and/or COPD, aged 52 to 76 yr (median and percentiles: 69 and 67 to 73 yr, respectively), were studied. Chronic bonchitis and COPD were defined according to the criteria of the American Thoracic Society as previously described in detail (12). Patients diagnosed as having COPD had an FEV_1 below 70% predicted, and displayed a 10% or smaller increase in their FEV_1 at the time of the procedure after an inhaled dose of 200 mg of albuterol. They were all smokers (30 to 85 pack/yr; mean ± SD: 38.2 ± 24.5 pack/yr). Patients were excluded if they had had a bronchial infection during the month preceding the study; no subject had received corticosteroids in any form during the 2 mo prior to the study. All chronic bronchitis patients had routine chest X-rays and computed tomographic (CT) scans. Patients with obvious emphysema, as assessed by routine chest X-ray and CT-scan, were excluded.

Sixteen subjects (age range: 27 to 39 yr; median and percentiles: 27.5 and 27 to 32 yr, respectively) were used as a control group. None of these subjects had ever suffered from asthma or chronic bronchitis. They had not had any bronchial or respiratory tract infection during the month preceding the study. All the subjects were lifelong nonsmokers, and their pulmonary function was within the normal range.

To assess whether smoking in itself, unrelated to chronic bronchitis or COPD, might influence elastase or α_1-PI levels, we measured the levels of both substances in induced sputum obtained from 10 healthy smokers (age range: 27 to 44 yr; median and percentiles: 33.5 and 28 to 40 yr). None of these subjects had ever suffered from asthma or chronic bronchitis. They had not had any bronchial or respiratory tract infection during the month preceding the study. Their pulmonary function was within the normal range.

The study was approved by the appropriate ethics committee, and the patients gave their informed consent for participation.

Induced Sputum Production and Processing

After giving written informed consent, each subject was submitted to spirometry. Induced sputum production and processing were done according to the methods of Fahy and colleagues (13), with slight modifications. Patients in a fasting condition for 20 min were exposed to an aerosol of 3% hypertonic saline solution early in the morning. The subjects were encouraged to cough throughout the procedure and regularly interrupted their inhalation of hypertonic saline in order to expectorate sputum into previously weighed, 50-ml sterile ampules. Subjects were asked to accurately wash their oral cavity with saline solution before expectorating sputum, as well as to blow their noses in order to minimize the salivary contamination of sputum. The aerosol was administered from an ultrasonic nebulizer (Fisoneb; Fisons Italchimici Spa, Rome, Italy) that generates particles with a median diameter of 2.5 μm and has an output of 1 ml/min. A sample of saliva was collected from all subjects before the sputum-induction procedure.

The volume of the induced sputum and saliva sample was determined, and an equal volume of dithiothreitol (DTT; Sigma Chemical Co., St. Louis, MO), diluted with saline solution to obtain a 0.1% concentration, was added. The samples were then mixed gently with a vortex mixer and were placed in a water bath at 37° C for 15 min to ensure complete homogeneization. The samples were removed from the water bath periodically for further brief, gentle vortex mixing. The homogenized sputum and saliva were centrifuged at 800 × *g* for 10 min to separate the supernatants from the cell pellet. The supernatants were then aspirated and frozen at −20° C for subsequent biochemical analysis.

The cell pellet was resuspended in saline solution, and the cell viability was assessed by Trypan blue exclusion. The cells were then cytocentrifuged (Cytospin 2; Shandon Instruments, Runcorn, UK) and stained with the Diff-Quik method (Merz-Dade, Dudingen, Switzerland) for differential cell counting. The slides were read blindly by two independent investigators (F.M. and A.M.), who counted at least 400 cells per slide. The number of squamous cells was subtracted from the total cell counts, and the differential cell counts were expressed as corrected percentages.

Biochemical Analysis of Sputum and Saliva

Total elastase was measured in samples of sputum and saliva with a homogeneous enzyme immunoassay (EIA) specific for human polymorphonuclear elastase (IMAC-Elastase Kit; Merck, Darmstadt, Germany) according to the package insert (14). Elastase levels over 20 μg/L can be measured with this EIA. The activity of active neutrophil elastase was determined according to the technique of Fujita and colleagues as follows (15): 200 μl of diluted sputum were added to 400 μl of the specific substrate methoxy-succinyl-ala-ala-pro-val-p-nitroanilide (Sigma), 0.2 mM, in 0.1 M 4-(2-hydroxyethyl)-1-piperazine-N′-2-ethanesulfonic acid (HEPES), 0.5 M NaCl, and 10% dimethyl-sulfoxide (DMSO) at pH 7.5. Purified human neutrophil elastase (ICN Products, Costa Mesa, CA) was used as a standard under the same conditions. After preincubation for 1 h at 37° C, the reaction was stopped with 200 μL of 1 N acetic acid. The absorbance of the p-nitroanilide product was measured at 410 nm, using a Beckman DU-65 spectrophotometer (Beckman, Mountain View, CA). To assess the effect of metalloelastases from bacterial or macrophage sources on the assay system, the inhibitory profile of active elastase was determined by 30 min of preincubation of the sample with 0.4 mM methoxysuccinyl-ala-ala-pro-val-chloromethyl ketone (CMK) (Sigma) and 50 mM ethylenediamine tetraacetic acid (EDTA) (16). At the end of the preincubation period, specific substrate was added, and the procedure described previously was followed. Levels of metalloelastases over 0.04 μg/ml can be measured with this technique.

α_1-PI and albumin in sputum and saliva samples were detected with a nephelometric assay (Beckman array protein system), using specific monoclonal antibodies provided by Beckman Immunochemistry Systems (17). The method used measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen–antibody reaction. The assay detected levels of α_1-PI and albumin that exceeded 2.8 μg/ml and 6 μg/ml, respectively.

Statistical Analysis

Results are expressed as medians and 25 to 75 percentiles. Statistical analysis was done with the Mann–Whitney U test to assess differences between groups. Spearman's rank correlation was calculated to assess the correlation between data.

RESULTS

Demographic Characteristics of Patients

The median ages of patients with asthma, of control subjects, and of healthy smokers were similar, but chronic bronchitis patients were significantly older than the subjects in the two other groups ($p < 0.005$). FEV_1 values of asthmatic patients ranged from 48% to 104% of predicted values (80, 65–92% of predicted) and in chronic bronchitis or COPD from 40 to 92% (median and percentiles: 80 and 73 to 91%). In addition, 2 of 10 patients in the bronchitis group had COPD, since their FEV_1 values were below 70%. All patients with chronic bronchitis or COPD had a normal total lung volume and diffusion capacity, thus excluding superimposed emphysema.

Total and Differential Cell Counts in Sputum and Saliva

The percentage of squamous cells was not significantly different in sputum samples obtained from control subjects, healthy smokers, asthmatic patients, or chronic bronchitis patients (Table 1). The corrected median total cell count was similar for control subjects, smokers, and asthmatic patients. It was higher in chronic bronchitis patients; however, the difference from the other groups was not statistically significant. The viability of sputum cells was 81.5% (range: 66 to 84.5%) in controls, 76.5% (range: 65 to 80%) in healthy smokers, 68.2% (range: 57.3 to 79.7%) in asthma and 72.5% (range: 61 to 78%) in chronic bronchitis. There was no significant correlation between the cell viability and elastase levels measured in

TABLE 1

CELL ANALYSIS OF SPUTUM SAMPLES

					p Value					
	Control Subjects	Smokers	Asthma	Chronic Bronchitis	p C/S	p C/A	p C/CB	p S/A	p S/CB	p A/CB
Squamous cells, %	27.5 (13.5–45)	28.5 (15–45)	28 (13–67.7)	16.5 (13–42)	NS	NS	NS	NS	NS	NS
Total cell counts, million cells/ml	1 (0.7–2.9)	1 (0.8–2)	1.2 (0.5–2.3)	2.2 (1.3–3.6)	NS	NS	NS	NS	NS	NS
Corrected cell counts, million cells/ml	0.6 (0.4–2.5)	0.7 (0.5–1.8)	0.5 (0.1–1.7)	1.7 (0.9–3.2)	NS	NS	NS	NS	NS	NS
Differential cell counts, %										
Macrophages	85 (75.5–94)	69.2 (66.8–79.5)	65 (31–78.7)	39 (17–72.6)	$p < 0.02$	$p < 0.002$	$p < 0.003$	NS	NS	NS
Neutrophils	14 (5–24)	30.1 (20–33)	32 (18.8–64.6)	58.3 (15.7–80)	$p < 0.03$	$p < 0.006$	$p < 0.03$	NS	NS	NS
Lymphocytes	0 (0–0.8)	0 (0–0.5)	0 (0–0.4)	0	NS	NS	NS	NS	NS	NS
Eosinophils	0	0	2 (1.4–2.3)	1.3 (0.5–2.3)	NS	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	NS
Epithelial cells	0 (0–0.5)	0.3 (0–0.8)	0 (0–1.6)	0.7 (0–5.3)	NS	NS	NS	NS	NS	NS

Definition of abbreviations: C = control subjects; S = smokers; A = asthma; CB = chronic bronchitis.
p Value by Mann–Whitney U test.

the four study groups. The percentage of neutrophils was significantly greater in chronic bronchitis patients, in asthmatic patients, and in healthy smokers than in control subjects; conversely, the difference between the asthma and chronic bronchitis patients was not statistically significant. The percentage of eosinophils was significantly higher in asthma and chronic bronchitis patients than in control subjects and healthy smokers; once again, asthmatic and chronic bronchitis patients did not show a significant difference from one another.

The total cell count in saliva was not significantly different in controls, healthy smokers, asthmatic patients, and chronic bronchitis patients (controls: 0.8 [range: 0.4 to 1] $\times 10^6$ cells/ml; healthy smokers: 0.7 [range: 0.5 to 0.9] $\times 10^6$ cells/ml; asthmatic patients: 0.7 [range: 0.3 to 1.6] $\times 10^6$ cells/ml; chronic bronchitis patients: 0.9 [range: 0.7 to 1] $\times 10^6$ cells/ ml); similarly, the median percentage of squamous cells in saliva was not significantly different in the four groups (controls: 99% [range: 97 to 100%]; healthy smokers: 98% [range: 97 to 100%]; asthma patients: 100% [range: 98 to 100%]; chronic bronchitis patients: 99% [range: 96 to 100%]).

Biochemical Analysis of Induced Sputum and Saliva

Total elastase and active elastase levels were found to be significantly higher in induced sputum obtained from asthmatic and chronic bronchitis patients than in sputum from control subjects ($p < 0.001$, Mann–Whitney U test) (Figure 1). In healthy smokers, the levels of both total and active elastase were significantly higher than in control subjects ($p < 0.03$ and $p < 0.002$, respectively) but significantly lower than in asthmatic ($p < 0.006$ and $p < 0.003$, respectively) and chronic bronchitis patients ($p < 0.006$ and $p < 0.002$, respectively). Active elastase activity was almost fully abolished (> 90%) by preincubation with the specific inhibitor CMK. There was a highly significant correlation between total and active elastase in sputum of patients with asthma (Rho = 0.9, $p < 0.0003$, Spearman's rank correlation) or chronic bronchitis (Rho = 0.9, $p < 0.004$). The percentages of active elastase were: 21% (range: 8 to 31%) for smokers; 32% (range: 25 to 39%) for asthma patients; and 42% (range: 24 to 47%) for chronic bronchitis patients.

In sputum from asthma and chronic bronchitis patients, the levels of both total and active elastase were significantly correlated with the percentage of neutrophils (asthma, total elastase: $p < 0.002$; active elastase: $p < 0.006$; chronic bronchitis, total elastase: $p < 0.02$; active elastase: $p < 0.02$; Spearman's rank correlation test) (Figure 2). In healthy smokers there was a significant correlation between the levels of total elastase and the percentage of neutrophils ($p < 0.03$, Spearman's rank correlation test). Moreover, the levels of both total and active elastase were found to be inversely correlated with FEV_1 values (%predicted) in both asthma (total elastase: $p < 0.04$; active elastase: $p < 0.04$) and chronic bronchitis patients (total elastase: $p < 0.04$; active elastase: $p < 0.02$; Spearman's rank correlation test) (Figure 3). Furthermore, the levels of active and total elastase correlated significantly with the absolute neutrophil counts in both asthma ($p < 0.006$ and $p < 0.003$, respectively, Spearman's rank correlation test) and chronic bronchitis patients ($p < 0.04$ and $p < 0.02$, respectively, Spearman's rank correlation test). In healthy smokers, the levels of total elastase correlated significantly with the absolute neutrophil counts ($p < 0.009$, Spearman's rank correlation test). Total elastase was also detected in saliva from the four study groups (normal subjects: 1.1 μg/ml [range: 0.4 to 2 μg/ml]; healthy smokers: 1.4 μg/ml [range: 0.8 to 1.9 μg/ml]; asthma patients: 1.3 μg/ml [range: 1 to 2.2 μg/ml]; chronic bronchitis patients: 2 μg/ml [range: 1.1 to 2.3 μg/ml]), but it did not correlate with the percentage of neutrophils in sputum samples. On the other hand, active elastase was undetectable.

The levels of α_1-PI were significantly higher in induced sputum obtained from asthmatic and chronic bronchitis patients than in that from normal subjects and healthy smokers (Figure 1). The difference between the two study groups was not statistically significant; however, in asthma but not in chronic bronchitis patients, a significant correlation was observed between the levels of α_1-PI and total elastase ($p < 0.02$, Spearman's rank correlation test). There was no significant correlation between α_1-PI levels and the absolute neutrophil counts in asthma and chronic bronchitis patients' sputum. The levels of α_1-PI in saliva were undetectable.

The ratio between total elastase and α_1-PI was 0.7 and 0.8 in asthma and chronic bronchitis patients' sputum, respectively. In control subjects' and healthy smokers' sputum, the levels of α_1-PI were always below the detection limit (2.8 μg/ml).

Albumin was found to be significantly increased in asthma (244 μg/ml [range: 118 to 445 μg/ml]) and chronic bronchitis patients' sputum (207 μg/ml [range: 146 to 324 μg/ml]) as compared with control subjects (84 μg/ml [range: 54 to 125 μg/ml]) and healthy smokers (115 μg/ml [range: 80 to 150 μg/ml]) ($p < 0.03$, Mann–Whitney U test). The median ratio between α_1-PI and albumin was 0.07 in both asthma and in chronic bronchitis patients' sputum. The median ratio between total elastase and albumin was 0.02 in control subjects, 0.03 in smokers, 0.04 in asthma patients, and 0.06 in chronic bronchitis patients (asthma versus control subjects: $p < 0.03$, Mann–Whitney U test; chronic bronchitis versus control subjects: $p < 0.02$, Mann–Whitney U test).

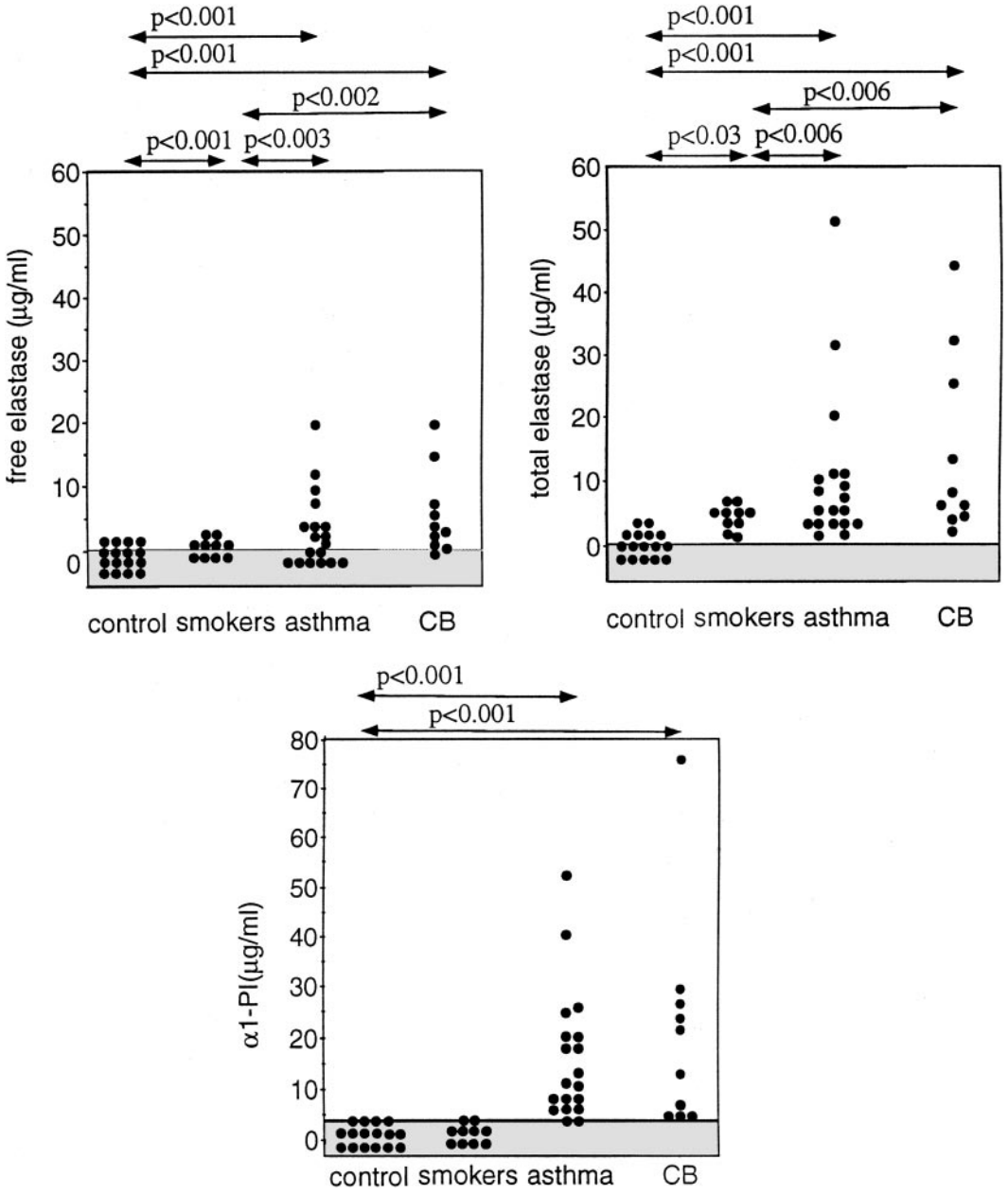

Figure 1. Levels of free elastase, total elastase, and α_1-antitrypsin (α_1-AT) in sputum obtained from control subjects, healthy smokers, asthmatic patients, and chronic bronchitis patients. Results are expressed as µg/ml. Statistical analysis was done with the Mann–Whitney U test.

DISCUSSION

This study shows that in induced sputum obtained from asthmatic and chronic bronchitis patients, the levels of both total and active elastase are significantly increased as compared with those in sputum from healthy smokers and control subjects, and are significantly correlated with the percentage of neutrophils. In addition, the levels of active and total elastase are inversely correlated with the degree of airway obstruction as assessed from FEV_1 values.

Chronic inflammation is a feature of asthma and chronic bronchitis and may be followed by healing or by structural alterations of the mucosa, such as epithelial damage (18) and connective-tissue destruction (19). Several mechanisms have been proposed as occurring in repair processes. Among them, the cellular activation of neutrophils, elastolysis, and elastosynthesis can play a crucial role. Polymorphonuclear neutrophils (PMN) can release a wide variety of enzymes, including extracellular matrix-degrading proteases and elastase, oxygen active radicals, and cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF-α), and IL-6 (18), and have therefore been implicated in chronic injury of the lung. Neutrophilic inflammation appears to be an important cellular component of induced sputum in asthma (20) and chronic bronchitis (21). In the present study we confirmed an increased number of neutrophils in sputum of patients with asthma or chronic bronchitis. Moreover, there was a significant correlation with FEV_1 levels in both diseases. With regard to asthma, the results obtained with sputum differ from those obtained with bronchoalveolar lavage fluid (BALF) or bronchial biopsies, in which neutrophils

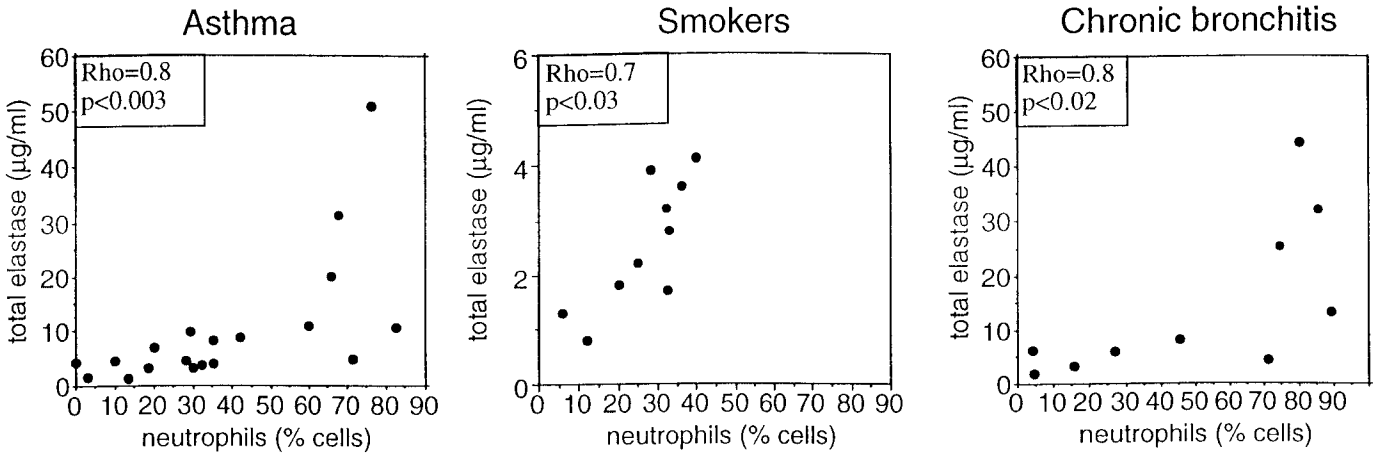

Figure 2. Correlation between levels of total elastase and percentage of neutrophils in sputum obtained from asthmatic patients, healthy smokers, and chronic bronchitis patients. Statistical analysis was done with Spearman's rank correlation test.

are rarely increased in number and are not correlated with the severity of the disease (22). The reasons for these differences remain to be fully clarified. Conversely, with regard to chronic bronchitis, the increased number of neutrophils in the sputum is in keeping with the results of (BALF) studies, which showed an increased number of neutrophils in comparison with that for normal subjects (22); this finding also confirms the results of previous studies showing a correlation between the severity of airflow limitation of patients with chronic bronchitis and neutrophilic inflammation in the airways (22).

Proteolytic enzymes have the potential to destroy many lung structures, provided that their activity exceeds the capacity of their natural inhibitors (5). In addition, inflammatory diseases such as chronic bronchitis are thought to be associated with an imbalance between proteinases and their inhibitors in the lung (6). Moreover, recent evidence has shown the presence of elastic fiber disruption in the bronchi of asthmatic individuals, supporting the concept of an imbalance between proteases and antiproteases in this disease (10). The results of the present study show increased levels of elastase in induced sputum from both asthmatic and chronic bronchitis patients as compared with healthy smokers and control subjects, and show that these levels are correlated with the severity of the diseases as assessed from FEV_1 values. We also detected an increase in α_1-PI levels in sputum samples obtained from asthmatic and chronic bronchitis patients as compared with control subjects, which depends on a compensatory response to high levels of active elastase. In addition, we found that in healthy smokers, the levels of both total and active elastase are significantly higher than in control subjects, suggesting that the smoking habit can contribute to the change in α_1-PI/elastase balance, and that such an imbalance is much more affected by the development of the inflammatory process associated with asthma or chronic bronchitis. Moreover, in asthma and chronic

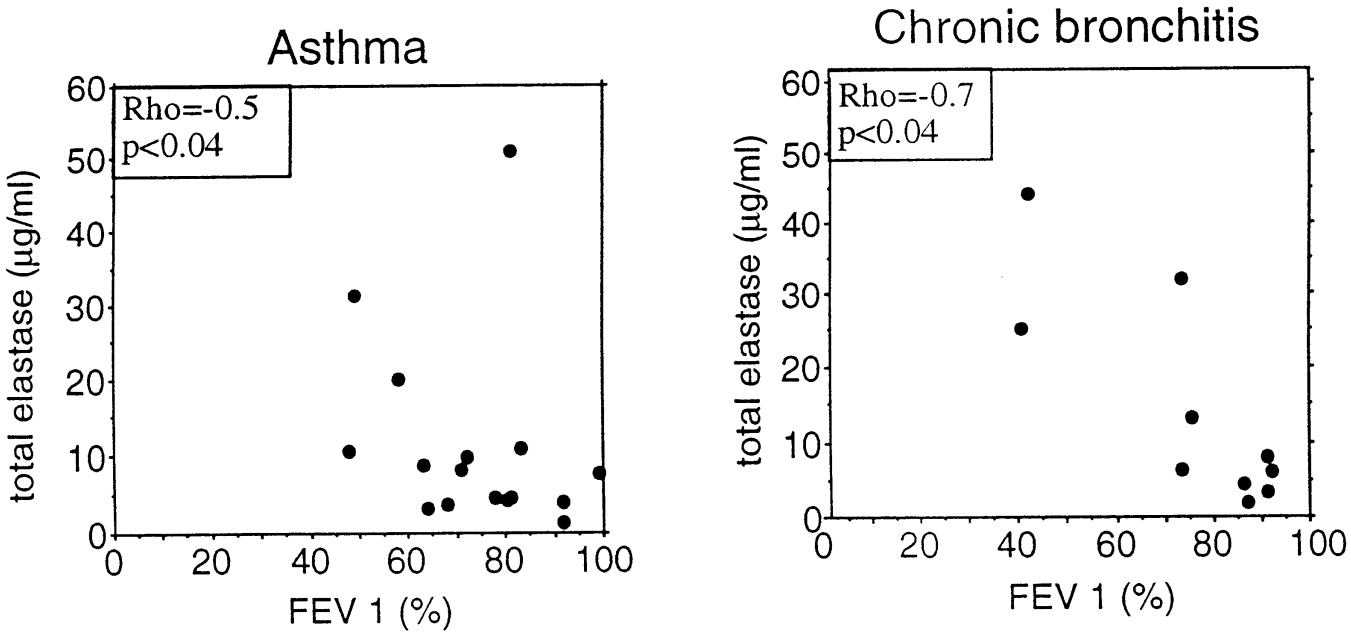

Figure 3. Correlation between levels of total elastase in sputum and FEV_1 values in asthmatic and chronic bronchitis patients. Statistical analysis was done with Spearman's rank correlation test.

bronchitis, the increased levels of elastase and α_1-PI might be partly due to an increased vascular permeability, as shown by the increased amounts of albumin measured in the sputum in both diseases. However, the increased levels of α_1-PI appear insufficient to counterbalance the increased levels of elastase, since in both diseases, high amounts of active elastase persisted. Several explanations for this can be provided. First, it is possible that the levels of elastase are so high as to preclude the inhibitory effects of α_1-PI, and second, α_1-PI can be inactivated by oxidant species (23), which are increased in asthma and chronic bronchitis. Third, α_1-PI can be inhibited by metalloproteinases, which are released in large amounts by neutrophils (24).

The imbalance between elastase and α_1-PI may play an important role in the degradation of components of the extracellular matrix and elastic fibers, as well as in the severity of both diseases. Neutrophil elastase can reproduce many of the pathologic features of asthma and chronic bronchitis, including mucous gland hyperplasia (25), excess mucus secretion (26), epithelial damage (23, 27), and connective-tissue destruction (6). In addition, neutrophil elastase can promote neutrophil recruitment in the lung by inducing production of IL-8 (28). Moreover, neutrophil elastase is involved in antigen-induced bronchoconstriction and airway hyperreactivity mediated by neutrophil accumulation and 5-lipoxygenase products in animal models, such as in guinea pigs (29). Elastase exposure has also been found to increase directed fibroblast migration through the extracellular matrix, a phenomenon that may play a role in development of the subepithelial fibrosis seen in inflammatory airway diseases such as asthma (30). Moreover, increased levels of active elastase may lead to disruption of the elastic fiber network of the bronchi, resulting in loss of the uniform intrapulmonary tension that is crucial for the even distribution of inspired air throughout the lung, since radial traction on the walls of the small airways and alveoli is required to maintain their patency during the inflation and deflation phases of ventilation (31). Alteration of the elastic fiber network also appears to have important pathophysiologic consequences in asthma, since most studies of moderately severe asthma in the stable state have found loss of elastic recoil and lung elasticity (32). Other possible consequences of elastic-fiber abnormality in asthmatic airways are an increased compressibility of the central airways in long-lasting asthma, resulting in loss of airway stability (33), and a lack of distensibility of asthmatic airways (34).

The major source of elastase in human lung is the neutrophil (3), but this enzyme may be released by several cell types in the airways, including activated macrophages and eosinophils (2). Our results suggest that neutrophils are the major cellular source of elastase, as indicated by the ability of CMK to almost completely abolish the elastase activity in the supernatants in our study, and by the significant correlation between the percentage of neutrophils and the levels of active and total elastase in sputum samples. Furthermore, the significant correlation between the levels of elastase and both the percentage of neutrophils and FEV_1 values in asthma and chronic bronchitis suggests a possible role for these cells in worsening of the clinical conditions of patients with both diseases. This hypothesis is further supported by the evidence that neutrophils isolated from chronic bronchitis (35) or asthmatic patients (20) appear more activated than neutrophils isolated from control subjects.

In conclusion, this study shows that in both asthma and chronic bronchitis there is an imbalance between elastase and its main inhibitor α_1-PI, and that this imbalance may play an important role in the pathogenesis of airway obstruction as well as in the development of many of the pathologic features characterizing these diseases.

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