Antileukoprotease in Sputum during Bronchial Infections*

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Production of sputum and concentrations of antileukoprotease (ALP) in sputum were serially measured in hypersecreting patients with recurrent or chronic bronchial infections. Patients with stable continuous mucoid (n = 4) or purulent (n = 5) secretions had constant sputum concentrations of ALP and a calculated daily production of 1.4 and 1.9 mg of ALP per 24 hours, respectively. In patients with overt recurrent bronchial infections, production of sputum and the ALP concentration were found to be negatively correlated during treatment with antibiotics (n = 14) and during the coming (n = 5) of an exacerbation. Daily production of ALP was rather constant in these groups (2.4 and 4.8 mg, respectively, per 24 hours). While ALP was not behaving like an acute-phase protein, bronchial infection appeared to be a determinant for production of ALP; however, between individual patients with comparable severity of disease, total production of ALP varied over tenfold. Therefore, bronchial infection is not the only factor in determining production of ALP.

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n 1972, an acid-stable low-molecular-weight protease inhibitor was isolated from bronchial secretions. The inhibitor had a distinct affinity to leukocyte-derived proteinases and proved to be responsible for most of the protease-inhibitory capacity of sputum. It has been called antileukoprotease (ALP) for these reasons. Morphologic studies have shown that ALP is generated by the serous cells of the glands in the respiratory epithelium and by several nonciliated epithelial cells in small bronchi and bronchioi, where subepithelial glands are absent.

It is most conceivable that the main task of ALP is to protect tissue against the destructive effect of leukocytic proteinases; however, at present, it is unknown to what extent the production of ALP varies within and between individuals, especially in relation to bronchial infections when proteinases are set free from leukocytes.

In patients with chronic obstructive pulmonary disease having substantial hypersecretion, we have measured the total daily production of sputum and its ALP content serially. Observations were done in the steady state and during the development and treatment of bacterial bronchial exacerbations.

MATERIALS AND METHODS

Clinical Data

Studies were done during 28 periods of measurable hypersecretion in 21 hospitalized patients (15 men and three women, aged 35 to 86 years). All had a history of chronic obstructive pulmonary disease, and most had reported recurrent or chronic purulent sputum for at least six months but often longer. In a number of them, bronchiec-
tatic lesions had been demonstrated by bronchography. Infections were caused by Hemophilus influenzae, Streptococcus pneumoniae, B. catarrhalis, E. coli, and Pseudomonas species. The patients were studied before, during, and after treatment with antibiotics. Usually they were also given bronchodilator drugs (sympathomimetics; theophylline; corticosteroids) by inhalation or the oral route. Patients who received reductant mucolytic drugs or anticholinergic drugs were excluded from this study.

Periods of hypersecretion studied were designated as A, B, C, or D, as follows: (A) continuous secretion of mucoid sputum, with negative culture of sputum (this group contained patients with simple chronic bronchitis or intrinsic asthma who were hypersecreting but had no bacterial bronchial infection during the observation); (B) continuous secretion of purulent sputum, with at least one positive culture of sputum without treatment with antibiotics (this group contained patients with bronchiectasis in whom the production of purulent sputum was permanent); (C) secretion of purulent sputum with at least one positive culture and subsequently receiving treatment with antibiotics (this group contained patients with exacerbations of recurrent infections, in whom the production of purulent sputum was temporary, in them, sputum was purulent at the beginning and usually mucoid at the end of the period studied); and (D) secretion of sputum, which was mucoid at the beginning of the period of study but turned to purulence and yielded at least one positive culture during the period of observation (these were patients who had completed an antibiotic course but developed a new infection within ten days thereafter).

Collection of Sputum

The patients were instructed not to swallow sputum and to collect all material brought up during coughing. Each morning after the first bronchial clearing, 24-hour samples were collected. After inspection by the naked eye (mucoid; mucopurulent; purulent) and weighing (in grams per 24 hours) the material was stored at −20°C. At least three samples were obtained during each period, the number varying from 3 to 20, covering periods up to three weeks. Usually, every other day's sample was used for ALP determinations.

Quantification of ALP in Sputum

Treatment of sputum and measurement of ALP were performed as described previously. In brief, 24-hour samples of sputum were thawed and homogenized by sonication. To the homogenized sam-

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CHEST / 89 / 5 / MAY, 1986 731
RESULTS

Observations in the Steady State

As shown in Figure 1A, in patients continuously producing mucoid sputum (group A; n = 4) or continuously producing purulent sputum (group B; n = 5), intrapatient variation of the daily concentration of ALP was small. In most of these patients, the ALP concentration was remarkably constant, even for weeks, especially when the daily production of sputum was relatively high; however, between individual patients, differences in mean sputum concentrations of ALP were considerable. Concentrations of ALP in purulent sputum (group B) varied more than in mucoid sputum (group A) and were often higher.

Results of measurements of the daily amount of sputum produced in groups A and B are given in Figure 1B. Variability of daily production of sputum production in the steady state was larger than variability of its ALP concentration; however, in seven out of nine patients, a negative Spearman rank correlation between the sputum concentration of ALP and the 24-hour production of sputum was found. This may indicate that ALP production tends to have a constant basic pattern in most patients and that a change in ALP concentration is mainly due to a dilution/concentration phenomenon, established by a change in the secretion of non-ALP-containing material.

Observations in Recurrent Bronchial Infections

To illustrate the material dealt with, data on production of sputum and ALP concentration in one patient gathered during 34 days are given in Figure 2. This patient had two periods during which an exacerbation
was treated with antibiotics and, between these, one period in which the coming of an exacerbation was observed. During these three periods, sputum production and ALP concentrations are evidently mirroring (r = -0.83; p = 0.0001).

Data on ALP concentration and sputum production obtained during 14 periods of treatment of a bacterial exacerbation in 13 patients (group C) are given in Figure 3 (parts I and II). Production of sputum diminished about threefold, and ALP concentration rose accordingly. The reverse was observed in group D (five patients) in whom an exacerbation was developing (Fig 3, parts III and IV). The changes in the sputum concentration of ALP and sputum production as depicted in Figure 3 for the groups were observed indeed within each individual.

When plotting the median daily production of sputum against the median ALP concentration of each group, a negative correlation was seen (Fig 4). This is further evidence for the trend found in patients who were in the steady state (Fig 1), suggesting that basic secretion of ALP is rather constant during the periods of 8 to 12 days studied and that ALP concentration is highly influenced by other secretions occurring during infections, acting as diluents.

Total Production of ALP

The daily production of ALP in groups A and B was calculated by multiplying the mean daily production of

![Graphs showing ALP concentration and sputum production over time.](image-url)
production (median value, 1.4 mg/24 hr). In group B (stable bronchiectatic patients with purulent sputum), higher values were found (median value, 1.9 mg/24 hr). At the beginning and the end of treatment for an exacerbation (group C) median values were 2.5 and 2.4 mg/24 hr, respectively; and at the beginning and the end of a period of upcoming infection, median values of ALP production were 4.8 and 4.6 mg/24 hr.

In all groups, the range was broad. Obviously, whether there was an active infection or not, and whether exacerbations were recent or not, these were not the only determinants of total ALP production. In every group, low and high producers of ALP occurred.

**Characteristics of Individual Patients**

We tried to sort out in what respects high producers of ALP distinguished themselves from low producers. Total production of ALP per 24 hours in each patient was plotted against age, sex, total tobacco consumption (pack-years), and tobacco consumption during the last five years but yielded no correlation. Neither the duration of signs of obstructive bronchial disease nor the frequency of purulent sputum during the foregoing two years was found to be related to total production of ALP.

**DISCUSSION**

The ALP in samples of sputum was measured by our ELISA technique. In this assay, homogenized acid-treated samples of sputum were tested after dilution in buffer containing rabbit serum. Under these conditions ALP-protease complexes are dissociated. So, the sputum level of ALP as measured by our assay represents both free and complexed ALP. Degradation of ALP in samples of purulent sputum, due to the presence of proteolytic activity, might result in unreliable measurements of the ALP level; however, ALP concentrations did not change when fresh sputum samples were kept at room temperature for more than 24 hours, indicating that ALP in these samples is very stable. Moreover, Tournier et al. observed that leukocytic elastase, which is present in purulent sputum at high concentration, protects ALP from degradation by *Pseudomonas aeruginosa* elastase, so it seems unlikely that in samples of purulent sputum, ALP is subject to proteolytic degradation.

In patients who had a steady-state continuous and measurable production of sputum, the ALP concentration of the secretions, as measured by our ELISA technique, was remarkably constant. Estimations of the amount of sputum produced were somewhat less reproducible. We have selected patients with daily expectoration and gave them careful instructions to collect all sputum in 24-hour aliquots. It is considered that bronchial secretions partly might have been swallowed and sometimes were deposited outside the col-

**Figure 4.** Median values of 24-hour production of sputum and ALP concentration in groups C and D. Days of sampling sputum during observation periods are indicated for each value.

sputum and the corresponding mean ALP concentration per patient, using the data in Figure 1. In groups C and D, mean sputum production and mean ALP concentration on the first two days and last two days of collection by each patient were used, according to the data in Figure 3. A survey of the so-calculated total ALP production in all periods studied is given in Figure 5.

Total ALP production varied from 0.2 mg/24 hr to 9.3 mg/24 hr. Patients who had mucoid sputum at the time of observation (group A) showed the lowest ALP production (median value, 1.4 mg/24 hr). In group B (stable bronchiectatic patients with purulent sputum), higher values were found (median value, 1.9 mg/24 hr). At the beginning and the end of treatment for an exacerbation (group C) median values were 2.5 and 2.4 mg/24 hr, respectively; and at the beginning and the end of a period of upcoming infection, median values of ALP production were 4.8 and 4.6 mg/24 hr.

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**Figure 5.** Calculated daily production of ALP in four groups of patients. For groups C and D, data of first (C', D') and last (C" and D") two days of observation period are given. Median values are indicated.
lecion bottle. Also, some saliva might have been added; however, negative correlations between the amount of 24-hour sputum production and ALP concentrations, in individual patients (Fig 1 and 2) as well as in groups (Fig 3), were found. This indicates strongly that such measurements reflected the real amount of sputum production in such a way that the total ALP production could be estimated.

The total daily production of ALP, calculated by multiplying ALP concentration and sputum production, varied greatly between but much less within individual patients. In the steady state, daily sputum production, ALP concentration, and calculated ALP production were rather constant. In patients being treated because of exacerbations of bacterial bronchial infections, ALP concentration and sputum production were negatively correlated, resulting also in a total daily ALP production which was rather constant in each patient. The increase of the amount of daily sputum when a bacterial infection is active seemed not to be correlated with an immediate increase of secretion by ALP-producing cells.

The normal volume of tracheobronchial secretions is estimated to range from 10 to 335 ml/day. Several cell types in the larger airways, such as mucous and serous glandular cells or goblet cells, and in the small airways (Clara cells and probably other nonciliated epithelial cells) participate in secretion, all together producing a heterogeneous product. How the several cell activities are regulated is hardly known, but even under normal conditions, the composition of sputum probably varies. In bronchial infections, microorganisms or their products may stimulate the production of secretions by causing exudation of serum and neutrophils or may stimulate sympathetic and parasympathetic nerve endings which govern the discharge of epithelial glands. In patients with chronic bronchitis, as caused by smoking, hypertrophy of mucous glands has been observed but not of serous glands. Antileukoprotease has been shown to be produced by serous but not by mucous elements of bronchial glands and by nonciliated epithelial cells in the membranous and terminal bronchioli. Our present finding that daily production of ALP is not influenced by changes in sputum production as caused by infections, at least for periods of one to two weeks, is in agreement with these data and would indicate that ALP-producing cells are not primarily involved in the anti-inflammatory response. Probably, exudation of fluid and neutrophils from the blood and secretion by mucous cells constitute the bulk of infectious secretions, and ALP in such material is very much diluted.

Obviously, our findings confirm another report that ALP does not behave like an acute-phase protein like $\alpha_1$-antitrypsin; however, mucoid sputum contained less ALP than purulent specimens (Fig 1), and patients with active infections (Fig 5) seemed to produce highest amounts of ALP. This would suggest that bronchial infections by and large do stimulate ALP production, be it long term and not within weeks. Probably it takes many months or years of frequent or chronic bronchial infections to stimulate ALP production in a considerable way.

While we have found some relation between infectious bronchial disease and ALP production, differences in ALP production between individual patients under much the same conditions with regard to infections were so great, over tenfold, that infections cannot be the only determinant for ALP production. Neither do age, smoking habits, or severity of bronchial obstruction seem to be important in this respect. Also, we were not able to demonstrate a correlation between ALP production and the duration of bronchial disease or the frequency of infections in the previous decades or smoking habits. Possibly, taking the history in retrospect was an insufficient device to gather such information. We are finally left with the intriguing question of why individual patients differ in ALP production. It is tempting to speculate that genetic factors may play a role.

Antileukoprotease is supposed to protect against proteolytic enzymes, especially leukocyte-derived elastase. It is unknown what damage is brought about by free elastase in the conducting airways. Studies in animals during which papain or elastase was deposited in the airways showed that emphysema can result, but morphologic damage to the bronchial wall is not mentioned in these studies; however, studies of mucociliary activity in vitro suggested diminished frequency of ciliary beats. Recently, it has been found that in dogs, papain-induced emphysema was accompanied by a decreased forced expiratory flow, which was about 50 percent disproportional to the amount of emphysema and would suggest a change in bronchial pressure area behavior resulting in a more peripheral location of the choke point. Furthermore, clinical experience has indicated that recurrent bronchial infections in the long term are often correlated with pathologic changes of the bronchial walls and bronchiectasis. It is possible that the development of such abnormalities is mediated by proteolytic enzymes, but other mechanisms may well be as important. The question arises as to whether a high production of ALP is correlated with high rate of protection. In our material, we could not find any evidence for higher ALP production being correlated with milder disease. To answer such questions, carefully designed prospective studies are needed.

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CHEST / 89 / 5 / MAY, 1986 735
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736

Antileukoprotease during Bronchial Infections (Dijkman, Kramps, Franken)