ELASTASE AND ACID PHOSPHATASE ACTIVITY IN THE BRONCHOALVEOLAR WASHINGS IN CHRONIC BRONCHITIS

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Changes in phagocytic cell function reflect the level of local protective factors of the tracheobronchial tree in bronchopulmonary pathology and may serve as an indicator of the effectiveness of treatment. A biochemical test which we used to evaluate the state of phagocytic cells is based on the ability of activated phagocytes to accumulate and excrete a whole range of substances, including hydrolytic enzymes.

The aim of this investigation was to study activity of hydrolytic enzymes (elastase and acid phosphatase) in the bronchoalveolar washings (BAW) of patients with chronic bronchitis in the course of medical treatment.

EXPERIMENTAL METHOD

A biochemical investigation of enzyme activity was conducted on 40 patients with chronic bronchitis over a period of time. The first portion of BAW was used for biochemical analysis. Material was taken at least 6-8 times from each patient with intervals of 2 days before, during, and after a course of treatment. The BAW were homogenized in a glass disintegrator, centrifuged for 15 min at 7000*g*, and enzyme activity and the total protein content were determined in the supernatant thus obtained. Acid phosphatase and elastase activity was determined with Na *p*-nitrophenyl ester of N-*tert*-butyloxycarbonyl-L-alanine respectively as substrates [2, 10]. Total protein was determined by Lowry's method [7]. A model of activation of phagocytes was created by the use of a culture of mouse peritoneal macrophages, isolated by the method in [8]. The following activating agents were used: *Salmonella typhimurium* lipopolysaccharide (LPS), from Sigma; *Salmonella typhi* Vi-antigen/polysaccharide, interferon γ-peptide, synthesized and generously provided by V. A. Maiorov (Institute of Immunology, Ministry of the Medical and Biological Industry of the USSR), hemagglutinin of influenza virus strain X-79 (H_3N_2) with mol. wt. of 78,000 daltons, isolated by the method in [6]. The activating agents were added to the culture of macrophages in a final concentration of: LPS and Vi-antigen 50 μg/ml, interferon peptide and glycoprotein of influenza virus 7 μg/ml. Samples without addition of any of these substances served as the control. Macrophages were incubated for 24 h at 37°C in an atmosphere of 5% CO_2, after which the cells were washed 3 times, harvested by centrifugation, and destroyed in a glass disintegrator. The homogenate was centrifuged and the resulting supernatant used to determine acid phosphatase and elastase activity by the methods mentioned above. A 0.05 M solution of di-isopropyl fluorophosphate ("Sigma") was used as serine proteinase inhibitor.

EXPERIMENTAL RESULTS

As a result of enzymic analysis of BAW from 40 patients with chronic bronchitis, before, during, and after a course of medical treatment (with antibiotics and mucolytics) definite general rules were discovered. In the case of effective treatment, acid phosphatase and elastase activity began to rise on the 7th-9th day after the beginning of treatment and reached a peak on the 10th-

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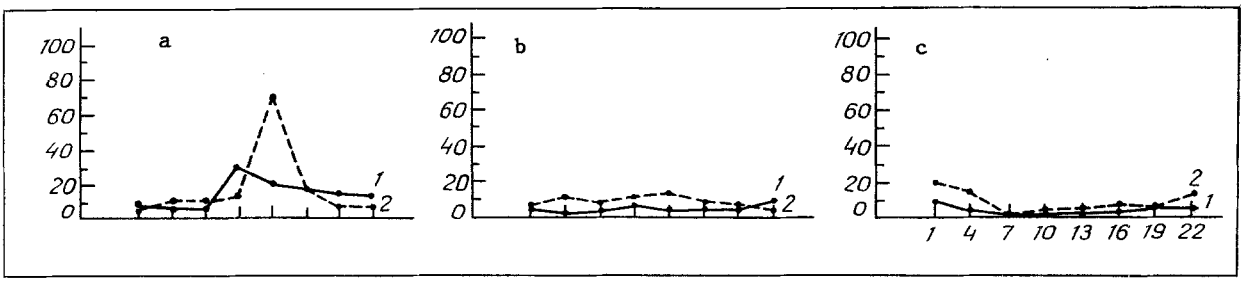

Fig. 1. Time course of acid phosphatase and elastase activity in BAW from patients with chronic bronchitis against the background of effective (a) and ineffective (b, c) treatment. Typical cases are illustrated. 1) Acid phosphatase activity, 2) elastase activity. Abscissa, time (in days); ordinate, enzyme activity (in μmoles p-nitrophenol/mg total protein).

TABLE 1. Intracellular Acid Phosphatase and Elastase Activity of Mouse Peritoneal Macrophages under the Influence of Various Activating Agents

Activating agent	Concn. of agent, μg/ml	Activity of	
		acid phosphatase	elastase
LPS	50	240	110
Vi-antigen	50	200	100
Interferon peptide	7	130	100
Glycoprotein of virus	7	260	98
Control	—	100	100

Legend. Activity of enzymes (in μmoles p-nitrophenol/ml extract) of experimental samples expressed as a percentage of control activity, taken as 100%.

13th day (Fig. 1a). The activity of these enzymes then declined, and approached the basic level. If treatment was ineffective, the activity of these enzymes was unchanged (Fig. 1b), or actually fell below the basal value (Fig. 1c). Comparison of average values (of 15 cases) of elastase and acid phosphatase activity in BAW from patients with chronic bronchitis on the 10th-30th day and on the 1st and 22nd days after the beginning of the investigation showed that the difference between them was statistically significant ($M \pm m$): for elastase 68.87 ± 6.32, 4.75 ± 1.36, and 11.0 ± 3.48, $p < 0.001$, and for acid phosphatase 41.75 ± 8.82, 8.12 ± 2.26, and 8.75 ± 2.33, $p < 0.01$, respectively. A differential cell count was carried out simultaneously on these same patients (by Senior Scientific Assistant O. V. Makarova, Institute of Human Morphology, Academy of Medical Sciences of the USSR), which showed that the predominant cells in BAW were macrophages and neutrophils, together accounting for 90-95% of the total number of cells.

Data in the literature indicate that the principal sources of acid phosphatase are activated macrophages and of elastase — neutrophils [4, 9]. To confirm this conclusion we used model experiments involving activation of mouse peritoneal macrophages in vitro by various activating agents. The results of these experiments, given in Table 1, show that all the activating agents used increased intracellular acid phosphatase activity was accompanied but had virtually no effect on elastase activity. The increase in intracellular acid phosphatase activity was accompanied, as was shown previously, by intensive excretion of this enzyme by the cell [1]. The absence of any change in elastase activity in the macrophages under the influence of the activating agents rules these cells out as a possible source of the elastase in BAW.

To discover the nature of the elastase in BAW of patients with chronic bronchitis, we used di-isopropyl fluorophosphate, a serine proteinase inhibitor. The elastase of neutrophils belongs to the group of serine proteinases and, unlike elastase of macrophages and metalloproteinases [5], it is inhibited by di-isopropyl fluorophosphate. Elastase activity was virtually absent in samples of BAW to which this inhibitor was added, possible evidence of its neutrophilic origin.

The study of the time course of elastase and acid phosphatase activity in BAW from patients with chronic bronchitis revealed a fundamentally new fact: the very brief peak-like increase in the activity of these enzymes in a focus of chronic inflammation, connected not only with an increase in the number of cells, but also an increase in their activity. This last conclusion was confirmed by calculating activity of the enzymes per 1000 cells. Specific activity of the enzymes, obtained in that way, showed a tendency to increase in the case of effective treatment. In cases when the cells did not respond with an increase in enzyme activity, treatment was of no benefit, probably because of its inappropriateness or as a result of a disturbance of local immunity.

The brief increase in hydrolytic enzyme activity which we found was due mainly to compensatory activation of phagocytic cells present in the focus of chronic inflammation and can be regarded as a prognostic sign of a favorable course of the disease. This phenomenon must be distinguished from the constant high level of enzyme activity in the inflammatory focus due to a massive inflow of cells from the bloodstream in the case of an unfavorable course of the disease. The character of the change in the level of hydrolytic enzyme activity in BAW of patients with chronic bronchitis is analogous to compensatory activation of the known proteolytic systems of blood plasma, namely the kallikrein-kinin, fibrinolytic, and complement systems [3].

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