

Alpha-1-Antitrypsin Content in the Serum, Alveolar Macrophages, and Alveolar Lavage Fluid of Smoking and Nonsmoking Normal Subjects

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ABSTRACT The content of alpha-1-antitrypsin in the serum, alveolar lavage fluid, and alveolar macrophages of smokers and nonsmokers was studied. Bronchoalveolar lavage was used to obtain alveolar fluid and macrophages from normal volunteers, and alpha-1-antitrypsin and albumin were measured using the electroimmunodiffusion technique. The serum level of inhibitor was not different between the two groups, while the total lavage fluid content of alpha-1-antitrypsin was increased in the smokers. The level of alpha-1-antitrypsin was also significantly greater ($P < 0.001$) in the alveolar macrophages of the smokers suggesting the possibility of chronically increased alveolar levels in the cigarette smoker as a possible protective mechanism against proteolysis.

INTRODUCTION

In 1963, Laurell and Eriksson first described the association of a deficiency of serum alpha-1-antitrypsin and pulmonary emphysema (1). This report stimulated much interest in the uninhibited proteolysis of the lung as a cause of the alveolar destruction and panlobular emphysema seen in these deficient subjects. The increased severity of emphysema at the lung bases in the homozy-

gously deficient patient has led Lieberman and Gawad to propose the polymorphonuclear leukocyte delivered to the lung bases via the circulation as the source of the proteases (2). A recent study in dogs suggests that intra-alveolar administration of proteolytic enzymes may be more important in the pathogenesis of experimental emphysema than i.v. delivery of enzymes (3). The alveolar macrophage as a source of proteolytic enzymes in relation to cigarette smoking in non-alpha-1-antitrypsin deficient subjects has also been proposed and studied (4-6).

The presence of enzyme inhibitors in lung lymphatic drainage (7) and sputum (8) has been documented, but those factors influencing either local production or transport of these inhibitors have not been elucidated. Cohen has reported the presence of alpha-1-antitrypsin by immunofluorescence in normal human alveolar macrophages and a diminished amount in the alveolar macrophages of a subject deficient in serum alpha-1-antitrypsin (9). In order to delineate the role of protease inhibitors in the lung and their relationship, if any, to the pathogenesis of emphysema, a quantitation of these proteins is necessary.

The following study was performed to evaluate more fully the presence of alpha-1-antitrypsin in the alveolar milieu of man.

METHODS

21 healthy volunteers were obtained from the inmate population of the Union Correctional Institution, Raiford, Fla.

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The subjects were divided into two groups based on smoking history: 11 smokers and 10 nonsmokers. The non-smoking category was limited to those stating that they had never smoked.

All subjects had a negative personal and family history of cardiorespiratory or liver disease. Each had a normal physical examination, chest X-ray, and routine spirometry.

Each volunteer underwent bronchoalveolar lavage by the method of Finley, Swenson, Curran, Huber, and Ladman (10) with cocaine and xylocaine topical anesthesia and meperidine, pentobarbital, and atropine i.v. sedation. Just before the lavage, 20 ml of venous blood was drawn. The serum was separated and frozen at -70°C . The fluid used in the lavage procedure was sterile normal saline. The total amount of fluid introduced into the lung averaged 350 cm^3 with a return of $100\text{--}300\text{ cm}^3$. The lavage material obtained was centrifuged at 250 g for 10 min and the fluid was decanted. The supernatant fluid was transported to the laboratory at 0°C and then concentrated to approximately 5 ml by pressure dialysis with an Amicon (Amicon Corp., Lexington, Mass.) ultrafiltration cell using a retentive filter for proteins of 1,000 mol wt or greater. This method consistently causes a loss of $55\%\pm 10\%$ of alpha-1-antitrypsin from the solutions which are being concentrated. Presumably, the alpha-1-antitrypsin sticks to the dialyzing membrane. The separated cells were transported to the laboratory at 0°C in Hanks's balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.), washed twice with normal saline, and counted with a standard hemocytometer. The lavage concentrate and cell bottom were then frozen at -70°C until analyzed.

The fluid concentrate was thawed and analyzed for the concentration of albumin and alpha-1-antitrypsin using a modification of the electroimmunodiffusing techniques of Lopez, Tsu, and Hyslop (11). The immunoelectrophoresis apparatus used has been described (12). A monospecific antiserum against albumin and alpha-1-antitrypsin was obtained from Behring (Behring Diagnostics, Sommerville, N. Y.), and alpha-1-antitrypsin reference standards in concentrations of 76 mg/100 ml, 190 mg/100 ml, and 380 mg/100 ml were obtained from Kallestad Laboratories (Kallestad Laboratories, Inc., Minneapolis, Minn.) (lot R360F010). The lavage fluid concentrate was diluted 1:10 for alpha-1-antitrypsin analysis and 1:40 for albumin. Serum samples were analyzed using the same technique at dilutions of 1:100 for alpha-1-antitrypsin and 1:1,000 for albumin. Samples and standard were placed in wells in $3\frac{1}{2}\times 4$ -inch agarose plates containing the appropriate antisera, and electrophoresis was carried out for 4 h with a 50 V, 45 mA power source. All samples were run in duplicate. The cell fractions, resuspended in normal saline, were homogenized with an ultrasonic homogenizer and the resultant homogenate was analyzed for alpha-1-antitrypsin in the same man-

ner. Cell homogenate protein content was determined by the method of Lowery, Rosebrough, Fan, and Randal (13). Statistical analysis of the differences noted in the data between the two groups of subjects was done using the non-paired Students *t* test.

RESULTS

The mean serum concentrations of alpha-1-antitrypsin and albumin and the mean pack years of smoking of the two groups are listed in Table I. The serum alpha-1-antitrypsin values ranged from 165 mg/100 ml to 355 mg/100 ml for smokers and 243 mg/100 ml to 314 mg/100 ml for nonsmokers. No statistically significant difference was observed in the serum data of these two groups. All alpha-1-antitrypsin values reported in this paper are dependent upon the accuracy of commercial standards used and quantitative comparison would be dependent upon the similarity of different standards. The standards used in this study were of one lot number only.

The mean lavage fluid concentration of alpha-1-antitrypsin and albumin are listed in Table II. The level of alpha-1-antitrypsin expressed per unit volume of fluid obtained from the lavage is considerably different between the two groups with a *P* value of 0.06. The concentration of albumin also tends to be increased in smokers but the difference also fails to reach statistical significance. With both the concentration of alpha-1-antitrypsin and albumin tending to be increased in smokers, the ratio of these two proteins remains almost identical to the ratio found in nonsmokers. The ratio of alpha-1-antitrypsin to albumin in the lavage fluid of both groups was greater than in the serum. The total amount of alpha-1-antitrypsin present in the lavage fluid from smokers was significantly greater (*P* < 0.05) than the total amount present in nonsmokers. Though this is equivocal data, it suggests that there is an increased alpha-1-antitrypsin concentration in the air spaces of the lung in cigarette smokers.

The levels of alpha-1-antitrypsin measured in the alveolar macrophage whole cell homogenate are shown in Table III. The differences between nonsmokers and smokers in cellular alpha-1-antitrypsin concentration, whether expressed on a per cell basis or per mg of cell protein, are highly significant with a *P* value of 0.001.

TABLE I
Serum Alpha-1-Antitrypsin (AAT) and Albumin Levels in Smokers and Nonsmokers

	Smoking	AAT	Albumin	AAT
	pack years	mg/100 ml	g/100 ml	mg/mg albumin
Nonsmokers (10)*	0	269 ± 6	4.50 ± 0.06	0.060 ± 0.001
Smokers (11)*	$16.6\pm 3.33\ddagger$	270 ± 16	4.36 ± 0.08	0.062 ± 0.003

* Number of subjects.

‡ Standard Error.

TABLE II
Alpha-1-Antitrypsin (AAT) and Albumin Concentration in Bronchoalveolar Lavage Fluid

	AAT	Albumin	AAT	Total AAT
	<i>mg/100 cm³</i>	<i>mg/100 cm³</i>	<i>mg/mg albumin</i>	<i>mg/lavage</i>
Nonsmokers (10)*	0.184±0.025‡	2.83±0.34	0.072±0.011	0.380±0.041
Smokers (11)*	0.306±0.057	3.69±0.55	0.076±0.007	0.592±0.079

* Number of subjects.

‡ Standard Error.

The cellular content of alpha-1-antitrypsin did not correlate with either serum alpha-1-antitrypsin level or the number of cigarettes smoked. However, it should be noted that two smokers had serum alpha-1-antitrypsin levels of 165 mg/100 ml and 177 mg/100 ml, and these two subjects had the second and third lowest macrophage alpha-1-antitrypsin levels in this group.

DISCUSSION

In the measurement of any substance in alveolar lavage fluid there are many theoretical, mathematical, and technical objections to this technique as a method of sampling the alveolar milieu (14), and the levels of alpha-1-antitrypsin in the concentrated lavage fluid cannot be considered quantitations because of the loss of protein during concentration. These objections may be abrogated somewhat by the presence of a control group, in this case the nonsmokers, and by relating the levels obtained to some other measurable constituent, i.e., albumin. Despite the problems involved of sampling and concentration, several conclusions may be drawn from this study.

Alpha-1-antitrypsin is assumed to be present in the lung in sufficient quantity in the normal individual to prevent proteolysis and in insufficient amount in the homozygously deficient patient. We have quantitated this protease inhibitor in the alveolar spaces and terminal airways of normal subjects and have found significantly elevated levels in the lungs of chronic cigarette smokers. This raises the interesting questions of why alpha-1-antitrypsin content is increased and what is the mechanism by which it is increased in cigarette smokers. The most obvious speculation is that an increase in protease inhibitor is present to perform its function of enzyme inhibition. This is circumstantially supported by the findings of increased protease activity in the alveolar macrophages of cigarette smokers (5, 6). We did not determine the functional state of the alveolar alpha-1-antitrypsin and it is possible that the globulin is complexed with enzyme or has been degraded. Lieberman has demonstrated that acid-degraded alpha-1-antitrypsin gives multiple peaks in the path of protein migration with the "rocket" technique of immunoelectrophoresis (15). In our study the immunoelectrophoretic patterns revealed a single

rocket suggesting that the alpha-1-antitrypsin in each test material was in the same physical state. Blondin, Rosenberg, and Janoff (16) have found an inhibitor of leukocyte elastase in the cytoplasm of human alveolar macrophages and this functionally active inhibitor could be alpha-1-antitrypsin. An important future study would be isolation and characterization of alpha-1-antitrypsin associated with alveolar macrophages.

The mechanism by which alpha-1-antitrypsin arrives in the alveolus is not known. Ganrot, Laurell, and Ohlsson have studied the concentration of proteins of various molecular weight in the plasma and lymph drainage of different organs in the dog and found similar lymph/plasma ratios of both alpha-1-antitrypsin and albumin in lung tissue (7). They suggested that their work supported previous studies indicating that capillary permeability of plasma proteins was inversely proportional to molecular size and they also concluded that lung capillaries were less selectively permeable than capillaries in other organs. If alpha-1-antitrypsin enters the alveolus by simple diffusion, it should arrive there in the same relative concentration as a molecule of similar size. In Tables I and II it can be seen that the concentrations of alpha-1-antitrypsin (mol wt 54,000) (17) expressed per mg of albumin (mol wt 67,000) is slightly greater in the lavage fluid than in the serum of our subjects. This might suggest that alpha-1-antitrypsin is actively transported into the alveolus or is locally produced but the simplest explanation would be a more selective diffusion because of the smaller molecular size. The increased alveolar

TABLE III
Alpha-1-Antitrypsin (AAT) Concentration in Pulmonary Alveolar Macrophage

	AAT	AAT
	<i>μg/10⁷ cells</i>	<i>μg/mg protein</i>
Nonsmokers (10)*	0.24±0.04‡	0.77±0.15
Smokers (11)*	2.55±0.58	4.26±0.87

* Number of subjects.

‡ Standard Error.

alpha-1-antitrypsin associated with cigarette smoking could be the result of increased capillary permeability associated with smoking, but we have no data which confirms such a view. However, our data does show that the ratio of alpha-1-antitrypsin/albumin in the lavage fluid remains the same in cigarette smokers as in nonsmokers, while the absolute concentrations increase. This favors increased passive diffusion as the mechanism by which alpha-1-antitrypsin is increased in alveoli of smokers. It would be important to determine the mechanism responsible for increasing alveolar alpha-1-antitrypsin because if this protein has a functional role of inhibiting proteolysis in the lung, abnormalities in alpha-1-antitrypsin transport could lead to an imbalance of proteolytic enzymes and inhibitor. This, then, could provide a pathophysiological explanation for the association of emphysema and cigarette smoking due to relatively uninhibited proteolysis of the lung.

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