Cigarette Smoking Induces Functional Antiprotease Deficiency in the Lower Respiratory Tract of Humans

Abstract. *Current concepts of the pathogenesis of emphysema suggest that it results from an imbalance of elastase and antielastase activity within the alveolar structures. Although emphysema that is associated with hereditary deficiency of serum α_1-antitrypsin conforms to this scheme, the major risk factor in the more common form of emphysema is cigarette smoking. A study was designed to evaluate the premise that cigarette smoking may be associated with an acquired, functional defect in lung α_1-antitrypsin. Determination of the antielastase activity of α_1-antitrypsin obtained from the lungs of smoking and nonsmoking individuals revealed a nearly twofold reduction in the functional activity of this elastase inhibitor in the lungs of cigarette smokers. These data suggest that cigarette smokers may lose some of the normal antielastase protective screen of the lower respiratory tract, making them more vulnerable to destructive lung disease.*

The alveolar structures are normally protected from elastolytic damage by the presence of antiproteases within the human lung (*1*). Studies of fluid from the epithelial surface of the lower respiratory tract, recovered through bronchoalveolar lavage of the lung, suggest that the major antiprotease of the alveolar structures is α_1-antitrypsin, a serum macromolecule that probably reaches the epithelial surface of alveoli by diffusion (*2*). The best evidence demonstrating the importance of α_1-antitrypsin in maintaining protease-antiprotease balance in the lower respiratory tract is the well-described association of severe, early-onset emphysema in persons with a hereditary deficiency of serum α_1-antitrypsin (*3*). In this disease, the α_1-antitrypsin produced by the liver has normal activity per unit weight, but is present in the circulation at a concentration insufficient to permit adequate protection of the alveolar structures from their elastolytic burden (*4*).

Although a severe deficiency of serum α_1-antitrypsin is clearly a risk factor for the development of emphysema, most individuals with emphysema have normal serum (and hence, lung) concentrations of this antielastase (*1*). For most people, it is cigarette smoking, not serum α_1-antitrypsin deficiency, that is the major risk factor for the development of emphysema (*5*). In fact, although a shift in protease-antiprotease balance in favor of the proteases has been widely presumed to be important in the pathogenesis of all forms of emphysema, except those resulting from α_1-antitrypsin deficiency, little direct evidence supports this hypothesis (*6*). Recent studies in vitro, however, have shown that cigarette smoke may interact with α_1-antitrypsin in such a way as to render it functionally inactive (*7*). A demonstration that this also occurs in vivo would add major support to the concept that the emphysema associated with cigarette smoking is the result of a protease-antiprotease imbalance within the lung. The purpose of this report is to demonstrate that cigarette smoking does result in an acquired deficiency of functional α_1-antitrypsin within the alveolar structures.

Functional and antigenic levels of α_1-antitrypsin were measured in fluid from the epithelial surface of the lower respiratory tract of cigarette smokers and nonsmokers. Two groups were studied. The first group consisted of ten healthy individuals with no evidence of lung disease uncovered by clinical, roentgenographic, and functional assessment. In this group were six nonsmokers (two

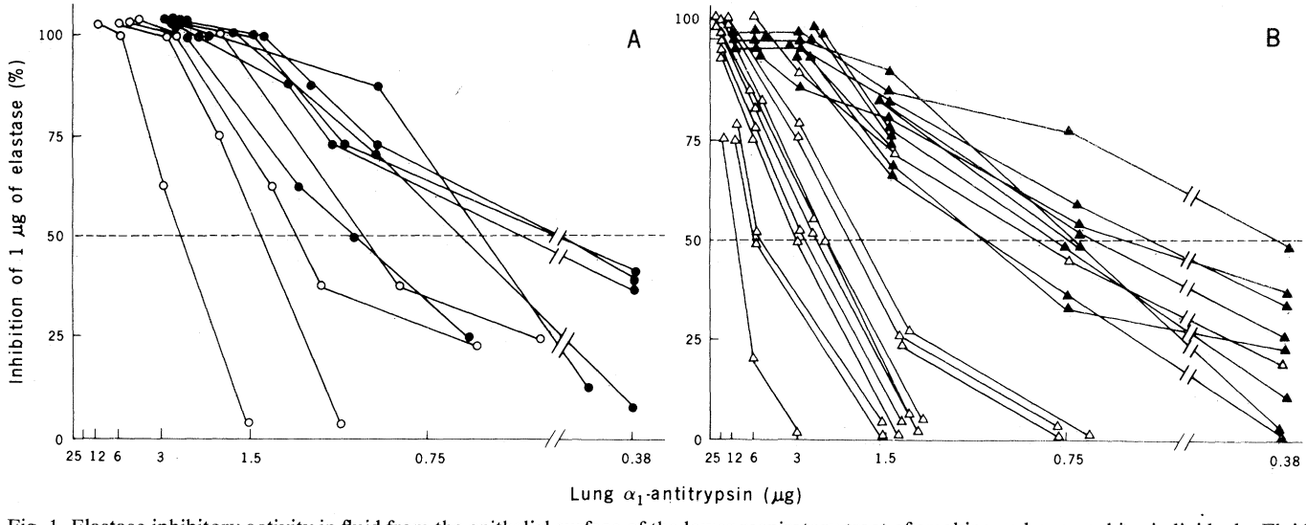

Fig. 1. Elastase inhibitory activity in fluid from the epithelial surface of the lower respiratory tract of smoking and nonsmoking individuals. Fluid was obtained by inserting a fiber-optic bronchoscope into a segmental bronchus (lingular or middle lobe). Five 20-ml portions of physiologic saline were injected through the bronchoscope; the lavage fluid was collected in a sterile vial by aspiration, separated from the cells by centrifugation at 500*g* for 5 minutes, and concentrated by pressure dialysis (Amicon UM2 membrane) to a volume of 1 ml. The concentrated samples were stored in the vapor phase of liquid nitrogen until assay. The amount of α_1-antiproteinase in each lavage sample was determined by rocket immunoelectrophoresis (*16*) in 1 percent agarose (*p*H 8.6) containing 1.5 percent monospecific goat antiserum to human α_1-antitrypsin (Atlantic Antibodies, Westbrook, Maine). Elastase inhibitory activity was ascertained by incubating dilutions of each lavage sample (at 25°C for 30 minutes) with porcine pancreatic elastase [1 μg per reaction mixture; specific activity, 2.7 μg of ^{3}H-labeled elastin, solubilized in 1 hour at 37°C (type ESFF, crystallized twice, Worthington Chemicals, Freehold, N.J.)]. For each lavage sample, an elastase inhibition curve was determined by using from 25 to 0.38 μg of α_1-antitrypsin per reaction mixture. The uninhibited elastase activity remaining in the reaction mixture was then assayed by adding the elastase-lavage mixture to an insoluble bovine elastin substrate which had been labeled by the sodium [^{3}H]borohydride method (*17*). Residual elastase activity was determined by the amount of [^{3}H]elastin-labeled peptides released from the substrate after 24 hours at 37°C. (A) Elastase inhibitory activity per microgram of α_1-antitrypsin in lung lavage fluid of asymptomatic smoking (○) and nonsmoking (●) individuals. (B) Elastase inhibitory activity per microgram of α_1-antitrypsin in lung lavage fluid of smoking (△) and nonsmoking (▲) individuals with idiopathic pulmonary fibrosis.

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males and four females; mean age, 31 ± 5 years) and four smokers (two males and two females; mean age, 35 ± 5 years). The smokers had smoked at least one pack per day for 27 ± 7 years (pack-years) (range, 15 to 40 pack-years). The second group consisted of 19 individuals with idiopathic pulmonary fibrosis, an interstitial lung disease characterized by chronic inflammation and fibrosis of the lower respiratory tract (*8*). In this group were eight nonsmokers (five males and three females; mean age, 45 ± 6 years) and 11 smokers (seven males and four females; mean age, 41 ± 7 years; smoking history, 37 ± 5 pack-years, with a range of 15 to 55 pack-years). All 19 individuals had biopsy-proven idiopathic pulmonary fibrosis in mid-course and fit the defined clinical, roentgenographic, and functional criteria for the disorder.

Evaluation of elastase inhibitory activity per unit of α_1-antitrypsin-containing lung fluid recovered indicated a marked reduction in inhibitory activity in the lavage fluid of cigarette smokers compared to that of nonsmokers (Fig. 1). This was true for both experimental groups. For the average healthy nonsmoker, 1.7 ± 0.15 μg of lung α_1-antitrypsin was needed to inhibit 100 percent of the activity of 1 μg of elastase. In marked contrast, for the average cigarette smoker, 2.8 ± 0.4 μg of lung α_1-antitrypsin was needed to inhibit 100 percent of the activity of 1 μg of elastase, implying that α_1-antitrypsin in the smoker's lung is only 62 percent effective as an antielastase ($P < .01$, t-test, two-tailed). Thus, for the healthy individuals, cigarette smoking was associated with an almost twofold reduction in effective activity of the major antiprotease of the alveolar structures, even though the healthy nonsmokers and healthy smokers had the same antigenic concentrations of α_1-antitrypsin in their lower respiratory tract (α_1-antitrypsin per unit of lung albumin: for nonsmokers, 51 ± 15 μg/mg; for smokers, 59 ± 20 μg/mg; α_1-antitrypsin in lavage fluid: for nonsmokers, 112 ± 30 μg per lavage; for smokers, 125 ± 40 μg per lavage; $P > .2$ in both comparisons). Similarly, the average smoker with idiopathic pulmonary fibrosis had more than a twofold reduction in the antielastase activity of the α_1-antitrypsin in his or her lungs compared to nonsmokers with the same disease. In the nonsmoking subjects, 2.1 ± 0.2 μg of lung α_1-antitrypsin was needed to inhibit 1 μg of elastase; in contrast, 5.0 ± 0.4 μg of lung α_1-antitrypsin recovered from the smokers was required to inhibit the same quantity of elastase ($P < .001$). Like the healthy, asymptomatic group, this was true even though smoking and nonsmoking individuals with idiopathic pulmonary fibrosis had similar antigenic concentrations of α_1-antitrypsin in their lower respiratory tracts ($P > .3$).

The demonstration of a twofold reduction in the effective activity of lung α_1-antitrypsin in cigarette smokers suggests that cigarette smoking causes an acquired, localized deficiency of α_1-antitrypsin. Several mechanisms may be responsible for this reduction in activity of α_1-antitrypsin:

1) Cigarette smoke may directly interact with lung α_1-antitrypsin in vivo and render it functionally inactive (*7*). In vitro, cigarette-smoke condensate (tar) and aqueous phase suppress the elastase-inhibiting effect of α_1-antitrypsin. Convincing evidence has been presented that cigarette smoke causes functional inhibition of α_1-antitrypsin by oxidizing a methionine residue near the elastase inhibitory site (*7, 9*).

2) Cigarette smoke may indirectly affect lung α_1-antitrypsin by virtue of its ability to recruit (*10*) and activate (*11*) blood neutrophils. There is evidence that cigarette smokers have neutrophils within their alveolar structures, whereas nonsmokers do not. Recently, experiments by Hunninghake *et al.* (*10*) have shown that cigarette smoke induces alveolar macrophages to liberate a low-molecular-weight chemotactic factor that activates neutrophils and attracts them to the lung; this may significantly tip the local protease-antiprotease balance in favor of proteases by increasing the protease burden [through release of elastase (*12*)] and by decreasing the functional activity of the α_1-antitrypsin in the microenvironment [through release of various oxidants that inactivate α_1-antitrypsin (*13*)].

3) Cigarette smoke may decrease the available functional α_1-antitrypsin by inducing the release of elastase from inflammatory cells in the lung. It has been reported that alveolar macrophages recovered from smokers, but not nonsmokers, release elastase in vitro (*14*). Also cigarette smoke apparently has a direct toxic effect on neutrophils, resulting in the release of elastase (*11*). At least in the case of neutrophil elastase, the free enzyme may complex with α_1-antitrypsin, leaving it antigenically intact but functionally inactive. However, preliminary studies to evaluate this possibility have suggested that this is unlikely, since α_1-antitrypsin in lavage samples from smokers maintains its normal electrophoretic mobility during bidirectional immunoelectrophoresis (*15*).

Independent of the mechanism by which cigarette smoking causes a reduction in the activity of α_1-antitrypsin, the findings presented in this report suggest an important parallel between the pathogenetic mechanism operative in the common form of emphysema associated with cigarette smoking and the rarer type of emphysema associated with an inherited deficiency of serum α_1-antitrypsin. In both disorders, the emphysema may be attributable to progressive proteolytic injury to the alveolar structures resulting from disruption of protease-antiprotease homeostasis within the lung, at least in part because of a decrease in the antiprotease activity in the lower respiratory tract.

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