

Risk factors for emphysema. Cigarette smoking is associated with a reduction in the association rate constant of lung alpha 1-antitrypsin for neutrophil elastase.

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Research Article

The increased risk of developing emphysema among individuals who smoke cigarettes and who have normal levels of alpha 1-antitrypsin (alpha 1AT) is hypothesized to result from a decrease in the antineutrophil elastase capacity of the lower respiratory tract alpha 1AT of smokers compared with nonsmokers. To evaluate this hypothesis we compared the time-dependent kinetics of the inhibition of neutrophil elastase by lung alpha 1AT from healthy, young cigarette smokers ($n = 8$) and nonsmokers ($n = 12$). alpha 1-antitrypsin was purified from lavage fluid using affinity and molecular sieve chromatography, and the association rate constant (k_{assoc}) for neutrophil elastase quantified. The k_{assoc} of smoker plasma alpha 1AT ($9.5 \pm 0.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$) was similar to that of nonsmoker plasma ($9.3 \pm 0.7 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, P greater than 0.5). In marked contrast, the k_{assoc} of smoker lower respiratory tract alpha 1AT was significantly lower than that of nonsmoker alpha 1AT ($6.5 \pm 0.4 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ vs. $8.1 \pm 0.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, P less than 0.01). Furthermore, the smoker lower respiratory tract alpha 1AT k_{assoc} was significantly less than that of autologous plasma (P less than 0.01). When considered in the context of the concentration of alpha 1AT in the lower respiratory tract epithelial lining fluid, the inhibition time for neutrophil elastase of smoker [...]

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Risk Factors for Emphysema

Cigarette Smoking Is Associated with a Reduction in the Association Rate Constant of Lung α 1-Antitrypsin for Neutrophil Elastase

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Abstract

The increased risk of developing emphysema among individuals who smoke cigarettes and who have normal levels of α 1-antitrypsin (α 1AT) is hypothesized to result from a decrease in the antineutrophil elastase capacity of the lower respiratory tract α 1AT of smokers compared with nonsmokers. To evaluate this hypothesis we compared the time-dependent kinetics of the inhibition of neutrophil elastase by lung α 1AT from healthy, young cigarette smokers ($n = 8$) and nonsmokers ($n = 12$). α 1-antitrypsin was purified from lavage fluid using affinity and molecular sieve chromatography, and the association rate constant (k_{assoc}) for neutrophil elastase quantified. The k_{assoc} of smoker plasma α 1AT ($9.5 \pm 0.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$) was similar to that of nonsmoker plasma ($9.3 \pm 0.7 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, $P > 0.5$). In marked contrast, the k_{assoc} of smoker lower respiratory tract α 1AT was significantly lower than that of nonsmoker α 1AT ($6.5 \pm 0.4 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ vs. $8.1 \pm 0.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, $P < 0.01$). Furthermore, the smoker lower respiratory tract α 1AT k_{assoc} was significantly less than that of autologous plasma ($P < 0.01$). When considered in the context of the concentration of α 1AT in the lower respiratory tract epithelial lining fluid, the inhibition time for neutrophil elastase of smoker lung α 1AT was twofold greater than that of nonsmoker lung α 1AT (smoker: $0.34 \pm 0.05 \text{ s}$ vs. nonsmoker: $0.17 \pm 0.05 \text{ s}$, $P < 0.01$). Consequently, for concentrations of α 1AT in the lower respiratory tract it takes twice as long for an equivalent amount of neutrophil elastase to be inhibited in the smoker's lung compared with the nonsmoker's lung. These observations support the concept that cigarette smoking is associated with a decrease in the lower respiratory tract neutrophil elastase inhibitory capacity, thus increasing the vulnerability of the lung to elastolytic destruction and thereby increasing the risk for the development of emphysema. (*J. Clin. Invest.* 1991. 87:1060-1065.)
Key words: protease • antiprotease • enzyme • oxidation

Introduction

Emphysema is a chronic disorder of the lower respiratory tract characterized by destruction of the walls of the alveoli (1, 2). When sufficient numbers of alveoli are involved, affected individuals develop dyspnea and hypoxemia that worsen with exertion (2, 3). The major cause of emphysema is cigarette smoking

(4). In this regard, autopsy studies have documented a much higher incidence of emphysema among cigarette smokers compared with nonsmokers and a correlation of the extent of emphysema with the degree of cigarette smoking (2, 5).

The pathogenesis of emphysema is hypothesized to result from an imbalance of proteases and antiproteases in the lower respiratory tract such that the level of proteases exceeds the antiprotease protection, allowing proteolytic destruction of the connective tissue matrix that provides the architectural support for the alveolar walls (6-8). This concept evolved as a consequence of two important observations: (a) the observation by Laurell and Eriksson (9) that deficiency of the enzyme α 1-antitrypsin (α 1AT)¹ is associated with the development of emphysema; (b) Gross et al. demonstrated the production of emphysema in normal rats by means of intratracheal injection of the proteolytic enzyme papain (10). In humans, the major protease of interest is neutrophil elastase, a broad-spectrum enzyme capable of cleaving all protein components of the connective tissue matrix of the alveolar walls (7, 11-14). Normally, neutrophil elastase is inhibited by α 1AT, a hepatocyte and mononuclear phagocyte-produced glycoprotein that serves as the major inhibitor of neutrophil elastase in blood and in the lower respiratory tract (7, 12, 15-17). In contrast, in α 1AT deficiency there is insufficient α 1AT available to inhibit the burden of neutrophil elastase presented to the lung, resulting in slow, unimpeded destruction of the alveolar walls (6, 7, 11, 12, 18, 19).

Despite the compelling evidence for the protease-antiprotease hypothesis to explain the pathogenesis of emphysema associated with α 1AT deficiency, less is known about why emphysema develops in cigarette smoking individuals who have normal levels of α 1AT. Although there is evidence that cigarette smokers have an increased burden of neutrophils in the lower respiratory tract compared with nonsmokers, the increase is modest, and the levels of α 1AT in the lungs of most cigarette smokers should be sufficient to adequately protect the alveolar walls (20-23). It has been hypothesized, however, that the α 1AT in the lower respiratory tract of cigarette smokers is less able to inhibit neutrophil elastase than that in the lungs of nonsmokers (23-25). This concept is based on the knowledge that the elastase inhibitory site of α 1AT is susceptible to oxidation and that cigarette smoke as well as alveolar macrophages recovered from smokers' lungs are able to oxidize α 1AT and render it less competent as an inhibitor of neutrophil elastase (17, 25-30).

Several groups have evaluated this hypothesis by attempting to determine what proportion of α 1AT molecules are completely functional inhibitors of elastase. Some of these studies

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1. Abbreviations used in this paper: α 1AT, alpha 1-antitrypsin; ELF, epithelial lung fluid.

have demonstrated that cigarette smoking is associated with a relative impotence of the anti-elastase screen (23, 31–33) while others have not (34–36). The inconclusive nature of these results is likely due to the limitation of available techniques that permitted only determining what proportion of the recovered α 1AT was completely impotent or completely functional (23, 31–37).

In this study, we have capitalized on the development of methods to purify sufficient amounts of α 1AT from the human lower respiratory tract to compare the time-dependent kinetics of the inhibition of neutrophil elastase by lung α 1AT from nonsmokers and smokers. In this context, we have been able to evaluate the hypothesis that cigarette smoking puts the individual at risk for the development of emphysema because the α 1AT molecules in the lower respiratory tract of the smoker take longer to inhibit a burden of neutrophil elastase than an equivalent number of α 1AT molecules in the lower respiratory tract of the nonsmoker.

Methods

Study population. The study population consisted of 12 nonsmokers and 8 current cigarette smokers. No individual in either group had evidence of disease by history, physical examination, chest x ray, and lung function tests. None were taking medication. The average age of the nonsmokers was 29 ± 2 yr and of the smokers was 30 ± 3 yr ($P > 0.5$). (All data are presented as mean \pm standard error of the mean; all statistical comparisons were made using the two-tailed Student's *t* test.) The average smoking history of the smokers was 15 ± 4 pack yr.

The nonsmoking group all had the α 1AT MM phenotype [pI M1M1, $n = 6$; pI M1M2, $n = 5$; pI M1M3, $n = 1$; phenotypes determined using isoelectric focusing and conventional criteria (11, 38)] as did the smoking group (pI M1M1, $n = 6$; pI M1M2, $n = 2$). The serum α 1AT levels (see below for methods) of the two groups were similar: nonsmoker, 245 ± 13 mg/dl (33.4 ± 1.7 μ M); smoker, 240 ± 25 mg/dl (32.8 ± 3.4 μ M), $P > 0.5$.

Bronchoalveolar lavage (BAL) of the two groups, carried out using 300 ml total vol as previously described (39), demonstrated similar values for the percent fluid recovered (nonsmoker $65 \pm 3\%$, smoker $64 \pm 3\%$, $P > 0.5$), for the recovery of albumin (nonsmoker 36.7 ± 5.2 μ g albumin/ml BAL, smoker 35.2 ± 3.9 μ g albumin/ml BAL, $P > 0.5$) and total volume of epithelial lung fluid (ELF) recovered (urea method [40], nonsmoker 1.6 ± 0.2 ml, smoker 1.4 ± 0.4 ml, $P > 0.5$). The total cells recovered were greater in the smoking group (nonsmoker $38 \pm 5 \times 10^6$, smoker $76 \pm 9 \times 10^6$, $P < 0.01$), but the cell differential was similar (nonsmoker: alveolar macrophages $93 \pm 1\%$, lymphocytes $4 \pm 1\%$, neutrophils $1 \pm 1\%$, eosinophils $0.1 \pm 0.1\%$; smoker: alveolar macrophages $96 \pm 1\%$, lymphocytes $3 \pm 1\%$, neutrophils $1 \pm 1\%$, eosinophils $0.1 \pm 0.1\%$, each cell type $P > 0.5$ nonsmoker compared with smoker).

α 1AT levels. The amounts of α 1AT in plasma were quantified using radial immunodiffusion (Calbiochem-Behring Corp., San Diego, CA). Levels of α 1AT in ELF recovered by bronchoalveolar lavage were quantified using an enzyme-linked immunoassay; the values in ELF were related to the volume of ELF recovered (see above) (40, 41). The quantification of amounts of α 1AT is complicated by the fact that the commercially available standard (Calbiochem-Behring) commonly used for clinical studies yields values for amounts of α 1AT that are higher than the true values (17, 42, 43). However, because clinical studies quantifying α 1AT serum levels over the past two decades have used such commercially available standards, the α 1AT values in the study will be presented based on both a commercial standard and on a true laboratory standard (44). The laboratory standard, a highly purified ($> 99\%$) preparation of α 1AT purified by the method of Laurell et al. (45), was isolated from serum of an individual homozygous for M1-type α 1AT, and quantified by amino acid analysis; this value was within 2% of the concentration determined using an extinction coeffi-

cient for α 1AT at 280 nm of 5.3 (42, 43). Values for the α 1AT concentrations presented as milligrams per deciliter are based on the commercial standard and those given as micromolar are based on the true laboratory standard. The time independent neutrophil elastase inhibitory activity of purified α 1AT from lavage fluid was determined in a titration assay as previously described (44). Levels of α 1AT in lavage fluid were related to the levels of albumin in the same sample. Albumin levels were measured in unconcentrated lavage supernatant with a direct competitive ELISA method, using human serum albumin (Calbiochem-Behring) and peroxidase-labeled human albumin, and goat IgG fraction anti-human albumin (Cappel Laboratories, Malvern, PA).

Purification of α 1AT from lavage fluid and plasma. α 1AT was purified from lavage fluid and plasma using modifications of the method of Sugiura et al. (46). Blood was collected in heparinized tubes on ice and the plasma immediately isolated by centrifugation. Lavage fluid was separated into its acellular and cellular components by centrifugation (300 g, 10 min) immediately after it was obtained and aliquots had been removed for cell number and differential (see above). Fresh acellular lavage fluid (100–150 ml) was then concentrated to 10–20 ml by pressure filtration at 4°C (YM-10 membrane; Amicon Corp., Danvers, MA). The α 1AT in plasma and concentrated lavage fluid (3-ml aliquots) was applied to a column of CNBr-activated Sepharose 4B beads (Pharmacia Fine Chemicals, Piscataway, NJ) with attached anti- α 1AT antibody (Accurate Chemical and Scientific Corp., Westbury, NY) and subsequently removed from the column with 0.1 M Na_2CO_3 , 0.5 M NaCl, pH 10.5. The α 1AT fraction was then concentrated by pressure filtration (YM-10) and then applied to a Sephadex G100 (for plasma) or a Superose 12 (Pharmacia) (for lavage fluid) molecular sieve equilibrated in 0.1 M Tris-HCl, pH 8.0, 0.5 M NaCl, 5 mM EDTA, 0.01% NaN_3 . The α 1AT peak was collected and then circulated for 12 h over a “negative selection” affinity column containing antibodies against human whole serum, albumin, prealbumin, α 2-macroglobulin, chymotrypsin, orosomucoid, IgG, IgA, IgM, C-1 esterase, and antithrombin III (all from Accurate Chemical and Scientific Corp.) (45). Purified α 1AT collected from this column was concentrated by pressure filtration (YM-10). The final concentration of purified α 1AT was determined by radial immunodiffusion as described above and stored in aliquots in liquid nitrogen vapor until used.

Quantification of the association rate constant of lung and blood α 1AT for human neutrophil elastase. The association rate constant (k_{assoc}) of α 1AT for human neutrophil elastase was determined by the method of Beatty et al. (47) with the minor modifications of Straus et al. (44). All measurements were made with the purified lavage and plasma α 1AT samples and with neutrophil elastase that had been titrated to quantify its activity as previously described (44). Briefly, equimolar amounts of human neutrophil elastase (Elastin Products, Pacific, MO) and active α 1AT (1 nM each) were incubated for varying times at 23°C in 0.1 M HEPES, pH 7.5, 0.5 M NaCl, and 0.1% Brij 35. Residual elastase activity at 0, 0.5, 1, 2, 3, 4, 5, 7, 30, and 60 min was quantified by adding the neutrophil elastase specific substrate methoxy-succinyl-alanyl-alanyl-prolyl-valyl-nitroanilide (1 mM, Sigma Chemical Co., St. Louis, MO). The k_{assoc} of the α 1AT was then calculated by the method of Beatty et al. (47) by plotting the residual elastase activity at each time point versus time. From the linear portion of the curve (the initial 4 to 5 min), the y intercept and slope were determined using least squares analysis. From this data the half-time of the reaction ($t_{1/2}$) was quantified as: (y intercept)/(slope). The association rate constant was then determined as: [(concentration of active neutrophil elastase in the reaction) \times ($t_{1/2}$)]⁻¹.

Estimation of the in vivo inhibition time of α 1AT for human neutrophil elastase. The relevance of the association rate constant of an inhibitor such as α 1AT for its substrate is critically dependent on the concentration of the inhibitor in the biologic fluid of interest (14, 15). Essentially, the “in vivo lung α 1AT inhibition time” is an estimate of the time required for five half-lives of the reaction of the α 1AT with a burden of elastase based on the amount of α 1AT present in the biologic fluid of interest. Using the formulation of Bieth (48, 49), the in vivo lung α 1AT inhibition time was quantified as: $5/[(k_{\text{assoc}}) \times (\text{concentration of } \alpha\text{1AT})]$.

tration of functional α 1AT in ELF)]. For each individual, the in vivo lung α 1AT inhibition time was quantified using that individual's values for ELF α 1AT k_{assoc} and ELF functional α 1AT concentrations. These individual in vivo inhibition times were used for the calculation of the group times for smokers and nonsmokers.

Results

α 1-Antitrypsin levels. The α 1AT levels in plasma of nonsmokers and of smokers were similar (nonsmokers 245 ± 13 mg/dl, smokers 240 ± 25 mg/dl, $P > 0.5$). The same was true in the epithelial lining fluid of the lower respiratory tract (nonsmokers 4.4 ± 0.5 μ M, smokers 4.0 ± 0.2 μ M, $P > 0.5$). In addition, no difference was observed in the amount of α 1AT in lavage fluid when referenced to the amount of albumin present (nonsmokers 52 ± 6 μ g α 1AT/mg albumin, smokers 49 ± 7 μ g/mg albumin, $P > 0.5$). Thus on the basis of the amounts of total α 1AT present in plasma or in ELF, normal nonsmokers and smokers cannot be distinguished. Evaluation of epithelial lining fluid α 1AT activity using the time-independent assay for activity revealed that nonsmokers' α 1AT was $78.4 \pm 3.7\%$ active while smokers' ELF was $62.3 \pm 3.0\%$ active ($P < 0.05$). However, although the value is lower in smokers than nonsmokers, in view of the long incubation times (2 h) this observation is not likely physiologically relevant, in contrast to the time-dependent (kinetic) comparison of α 1AT activity of nonsmokers' and smokers' ELF α 1AT, which more accurately reflects what is likely ongoing in vivo (see references 48, 49 for a discussion of kinetic versus nonkinetic analyses in regards to protease inhibitors).

Comparison of the association rate constant of nonsmoker and smoker blood and lung α 1AT for neutrophil elastase. There was no difference in the ability of α 1AT in the plasma of nonsmokers and smokers to inhibit neutrophil elastase in a time-dependent fashion. In this regard, the k_{assoc} of plasma α 1AT for neutrophil elastase for nonsmokers was $9.3 \pm 0.7 \times 10^6$ $\text{M}^{-1}\text{s}^{-1}$ and for smokers was $9.5 \pm 0.5 \times 10^6$ $\text{M}^{-1}\text{s}^{-1}$, $P > 0.5$. Thus, when in the vascular compartment, the α 1AT molecules in nonsmokers has a similar potential to inhibit neutrophil elastase as do the plasma α 1AT molecules in cigarette smokers.

In marked contrast to the vascular compartment, comparison of the k_{assoc} for neutrophil elastase of α 1AT purified from lavage fluid was significantly lower for smokers ($6.5 \pm 0.1 \times 10^6$ $\text{M}^{-1}\text{s}^{-1}$) than nonsmokers ($8.1 \pm 0.1 \times 10^6$ $\text{M}^{-1}\text{s}^{-1}$, $P < 0.01$) (Fig. 1). Thus, for cigarette smokers, on the average, the α 1AT molecules on the epithelial surface of the organ to which cigarette smoke is directed had a k_{assoc} for neutrophil elastase that was 20% less than the α 1AT molecules at the same site in nonsmokers.

Estimates of in vivo relevance. The in vivo relevance of the reduced k_{assoc} of smoker lung α 1AT for neutrophil elastase is vividly conceptualized by estimating the in vivo α 1AT lung inhibition time for neutrophil elastase (Fig. 2). In this regard, for the levels of functional α 1AT found in ELF for each individual, the k_{assoc} of smoker lung α 1AT translates into an in vivo inhibition time of 0.34 ± 0.01 s, while the k_{assoc} of nonsmoker lung α 1AT translates into an inhibition time of 0.17 ± 0.01 s, a value twofold higher for smokers. Thus, for the same burden of neutrophil elastase in the ELF of the lower respiratory tract, the population of α 1AT molecules in the smoker ELF would take twice as long to effectively inhibit the elastase burden.

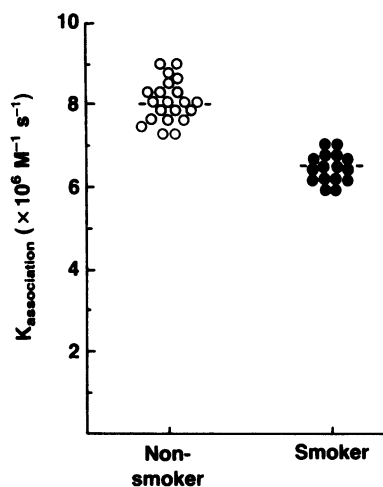


Figure 1. Comparison of the association rate constant of nonsmoker and smoker lung α 1-antitrypsin for neutrophil elastase. α 1AT purified from lavage fluid was incubated with neutrophil elastase for varying indicated times and the k_{assoc} of the α 1AT for neutrophil elastase quantified. Shown are data from 21 lung α 1AT samples from 12 nonsmokers (○) and 16 samples of lung α 1AT

of 8 smokers (●). Each determination represents the average of triplicate determinations. The horizontal line is the mean for the entire population.

Discussion

The alveolar walls are delicate structures comprised of epithelial cells lining a thin connective tissue interstitium surrounding the pulmonary capillaries (11, 50). The interstitium plays a critical role in lung function in that it defines the overall architecture of the lower respiratory tract, and modulates the mechanical properties of the lung parenchyma during respiration (50, 51). If the integrity of the connective tissue matrix of the alveolar walls is lost, the alveolar wall is destroyed; when this occurs in sufficient numbers of alveoli, the consequence is the clinical disorder emphysema. In this regard, emphysema is conceptualized as developing in circumstances where the mechanisms protecting the integrity of the alveolar connective tissue matrix are overwhelmed, either because the processes destructive to the alveolar walls are exaggerated and/or the defenses of the lower respiratory tract that protect the alveolar walls are deficient (6–8, 11, 12, 18, 19).

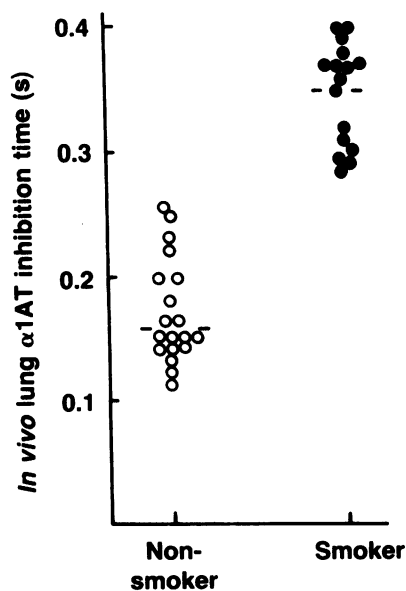


Figure 2. In vivo lung α 1-antitrypsin inhibition time for neutrophil elastase. The in vivo inhibition time is an estimate of the time required to inhibit neutrophil elastase relevant to the concentration of the α 1AT present (see Methods). Data are shown for 19 samples from 11 nonsmokers (○) and 16 samples from 8 smokers (●). Each determination represents the average triplicate determinations. The horizontal line represents the mean for the entire population.

Although a variety of neutral proteases have been evaluated as potential candidates as causing the alveolar wall destruction associated with cigarette smoking, most attention has focused on neutrophil elastase, a 29–33 kD glycoprotein capable of cleaving all major connective tissue proteins that form the connective tissue matrix of the alveolar walls (14). Consistent with this concept, the lungs of cigarette smokers are known to be exposed to an exaggerated burden of neutrophil elastase (20, 21, 52, 53). In the normal human lung, the alveolar walls are protected against the destructive potential of this neutrophil elastase burden by α 1AT (6, 7, 11, 22), an antiprotease that has such a high association rate constant for neutrophil elastase that the free enzyme chooses to bind to the active site of the α 1AT rather than attack the connective tissue components of the alveolar walls (15, 47). In this regard, the observation in this study, that the k assoc of the α 1AT in the lower respiratory tract of cigarette smokers is reduced compared with normal, provides important evidence supporting the concept that the increased risk for the development of emphysema associated with cigarette smoking is linked to a relative inability of the normal protective mechanisms in the lower respiratory tract to provide a sufficient antineutrophil elastase screen to prevent the increased burden of neutrophil elastase in the smoker's lung to protect fragile alveolar walls.

This study expands upon the evaluation by several groups of investigators (23, 27, 33, 37) of the static properties of the antielastase screen of the human lower respiratory tract. All of these previous studies, however, were limited by the available technology that permitted only asking what proportion of the molecules protecting the lower respiratory tract against neutrophil elastase were completely functional or not. Wewers et al. found a 40% decrease in the time-dependent inhibition of neutrophil elastase by bronchoalveolar lavage fluid (37); however, since bronchoalveolar lavage fluid is a mixture of elastase inhibitors, including secretory leukoprotease inhibitor and small quantities of α 2-macroglobulin in addition to α 1AT, it is unclear whether the effect that was seen was due to reduced neutrophil elastase inhibitory capacity of α 1AT or to other factors present in lavage fluid. Capitalizing on the development of methods to purify the small quantities of α 1AT that can be recovered from the human lower respiratory tract (46), and combining this with the methods developed by Beatty et al. (47) to evaluate the time-dependent kinetics of the inhibition of neutrophil elastase by α 1AT, we have been able to ask whether the α 1AT molecules in the lungs of smokers are similar or different from the α 1AT molecules in nonsmokers' lungs in their ability to provide an attractive target to adsorb the burden of neutrophil elastase in the lower respiratory tract. Using this approach, the answer is clear: for a similar amount of neutrophil elastase, the antineutrophil elastase screen of the lower respiratory tract of the cigarette smoker will take twofold longer to inhibit the neutrophil elastase than the lower respiratory tract antineutrophil elastase screen of the nonsmoker. Put in the context of the knowledge that cigarette smoking brings with it an exaggerated burden of neutrophil elastase to the alveolar structures (20, 21, 52, 53), this observation of the relative time-dependent kinetic impotence of the lung α 1AT in the smokers' lung suggests that smokers are at greater risk for having uninhibited neutrophil elastase in the lower respiratory tract that could potentially destroy the alveolar walls.

The importance of the deficiency in the time-dependent kinetics of smokers' lung α 1AT can be appreciated by compari-

son of the risk of developing emphysema among cigarette smokers with normal α 1AT levels to the emphysema risk of individuals with the α 1AT phenotype MZ, who have serum α 1AT levels as low as 18 μ M. MZ heterozygotes who do not smoke are not a greater risk for emphysema than nonsmoking MM homozygotes (54, 55) despite their markedly lower levels of α 1AT. In sharp contrast are MM individuals who smoke, in whom the risk of emphysema is significantly increased. This observation highlights the importance of diminished function of α 1AT within the lung; the observed decrease of 20% in the k assoc and an increase of 40% in the inhibition time for neutrophil elastase suggests that this functional deficiency is not simply a finding of only statistical significance, but is clinically significant as well. This study was directed at only evaluating the time-dependent kinetics of the antineutrophil elastase screen of the lower respiratory tract of smokers, not determining why the α 1AT molecules in the lower respiratory tract are less able to inhibit neutrophil elastase. There are, however, a number of observations that suggest that the mechanism is, at least in part, associated with oxidation of the Met³⁵⁸ residue at the active inhibitory site of the α 1AT molecule. First, the gas phase of tobacco smoke is a rich source of oxidizing agents (27, 29). Second, alveolar macrophages recovered from the lower respiratory tract of cigarette smokers are spontaneously releasing exaggerated amounts of oxidants such as superoxide anion and hydrogen peroxide (30, 56, 57). Third, when α 1AT is exposed to cigarette smoke (24, 27, 29) or to activated alveolar macrophages or neutrophils (58–61), the active inhibitory site of the α 1AT is oxidized. Fourth, in vitro studies have demonstrated that alveolar macrophages recovered from smokers' lungs spontaneously release sufficient oxidants to oxidize the active site of the amounts of α 1AT present in the lower respiratory tract (30). Fifth, when the active inhibitory site of α 1AT is oxidized, the association rate constant for neutrophil elastase is reduced (47). Sixth, direct analysis of the methionine residues in α 1AT recovered from the lower respiratory tract of smokers has demonstrated it is in the sulfoxide form, a state of methionine associated with being exposed to an oxidant burden (26, 31). The burden of oxidants associated with cigarette smoking is not uniformly distributed in the lung, with the result that lung α 1AT represents a mixture of oxidized and normal nonoxidized α 1AT molecules. Regional ventilatory inhomogeneity due to localized airways disease, or simply due to the normal preferential ventilation to lower rather than upper lung zones means that the contribution to the lung oxidant burden of inhaled cigarette smoke is not evenly distributed throughout the lung. Additionally, in smokers, localized areas of macrophage accumulation (62) can yield local milieus in which inflammatory cell oxidant production results in a high concentration of oxidants relative to other lung areas. Other potential factors, such as the availability of antioxidants and transudation of normal nonoxidized α 1AT from plasma into the alveolar space may influence the status of α 1AT within the lower respiratory tract. These variables will have the effect that not all α 1AT molecules within the lung will be exposed to the same oxidant burden and therefore will not be oxidized to the same degree, thus leading to an overall lung α 1AT molecular population composed of a mixture of oxidized and nonoxidized α 1AT.

Since the α 1AT obtained by lavage is a mixture of both oxidized and nonoxidized molecules, and it is not possible to separate the two, evaluation of the functional relevance of the

effect of oxidation on the ability of lower respiratory tract α 1AT to protect lung macromolecules such as elastin or collagen is not feasible. It has been shown, however, that oxidized α 1AT is essentially unable to inhibit elastolysis of lung elastin (63). Thus, on the basis of what is known, it is reasonable to hypothesize that in regions of the lung where oxidized α 1AT and neutrophils are present, conditions likely permit elastolytic degradation to occur. That this may occur in a regional pattern rather than globally throughout the lung is consistent with the disease pattern of emphysema in cigarette smokers, in which tissue destruction occurs in a nonuniform manner.

Thus, the relative impotence of the α 1AT molecules in the smoker lung is not an "all or none" phenomenon; due to the complex interplay of ventilation, oxidants, and antioxidants, and α 1AT turnover, lung α 1AT is not in a completely oxidized form. Finally, the fact that the relative impotence of the α 1AT molecules in the smoker is confined to the lung and not observed in plasma, is consistent with the knowledge that the burden of oxidants associated with the lung is localized to the lung.

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