Risk Factors for Emphysema

Cigarette Smoking Is Associated with a Reduction in the Association Rate Constant of Lung α1-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 α1-antitrypsin (α1AT) is hypothesized to result from a decrease in the antineutrophil elastase capacity of the lower respiratory tract α1AT of smokers compared with nonsmokers. To evaluate this hypothesis we compared the time-dependent kinetics of the inhibition of neutrophil elastase by lung α1AT from healthy, young cigarette smokers (n = 8) and nonsmokers (n = 12). α1-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 α1AT ($9.5\pm0.5 \times 10^6$ $M^{-1}s^{-1}$) was similar to that of nonsmoker plasma ($9.3\pm0.7 \times 10^6$ $M^{-1}s^{-1}$, $P > 0.5$). In marked contrast, the k assoc of smoker lower respiratory tract α1AT was significantly lower than that of nonsmoker α1AT ($6.5\pm0.4 \times 10^6$ $M^{-1}s^{-1}$ vs. $8.1\pm0.5 \times 10^6$ $M^{-1}s^{-1}$, $P < 0.01$). Furthermore, the smoker lower respiratory tract α1AT k assoc was significantly less than that of autologous plasma ($P < 0.01$). When considered in the context of the concentration of α1AT in the lower respiratory tract epithelial lining fluid, the inhibition time for neutrophil elastase of smoker lung α1AT was twofold greater than that of nonsmoker lung α1AT (smoker: 0.34±0.05 s vs. nonsmoker: 0.17±0.05 s, $P < 0.01$). Consequently, for concentrations of α1AT 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 α1-antitrypsin (α1AT)[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 α1AT, 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 α1AT deficiency there is insufficient α1AT 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 α1AT deficiency, less is known about why emphysema develops in cigarette smoking individuals who have normal levels of α1AT. 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 α1AT 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 α1AT 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 α1AT is susceptible to oxidation and that cigarette smoke as well as alveolar macrophages recovered from smokers' lungs are able to oxidize α1AT 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 α1AT molecules are completely functional inhibitors of elastase. Some of these studies

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1. *Abbreviations used in this paper:* α1AT, 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 α1AT 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 α1AT from the human lower respiratory tract to compare the time-dependent kinetics of the inhibition of neutrophil elastase by lung α1AT 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 α1AT molecules in the lower respiratory tract of the smoker take longer to inhibit a burden of neutrophil elastase than an equivalent number of α1AT 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±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 α1AT 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 α1AT levels (see below for methods) of the two groups were similar: nonsmoker, 245±13 mg/dl (33.4±1.7 μM); smoker, 240±25 mg/dl (32.8±3.4 μM), $P > 0.5$.

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±3%, smoker 64±3%, $P > 0.5$), for the recovery of albumin (nonsmoker 36.7±5.2 μg albumin/ml BAL, smoker 35.2±3.9 μg 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±5 $\times 10^6$, smoker 76±9 $\times 10^6$, $P < 0.01$), but the cell differential was similar (nonsmoker: alveolar macrophages 93±1%, lymphocytes 4±1%, neutrophils 1±1%, eosinophils 0.1±0.1%; smoker: alveolar macrophages 96±1%, lymphocytes 3±1%, neutrophils 1±1%, eosinophils 0.1±0.1%, each cell type $P > 0.5$ nonsmoker compared with smoker).

α1AT levels. The amounts of α1AT in plasma were quantified using radial immunodiffusion (Calbiochem-Behring Corp., San Diego, CA). Levels of α1AT 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 α1AT is complicated by the fact that the commercially available standard (Calbiochem-Behring) commonly used for clinical studies yields values for amounts of α1AT that are higher than the true values (17, 42, 43). However, because clinical studies quantifying α1AT serum levels over the past two decades have used such commercially available standards, the α1AT 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 α1AT purified by the method of Laurell et al. (45), was isolated from serum of an individual homozygous for M1-type α1AT, and quantified by amino acid analysis; this value was within 2% of the concentration determined using an extinction coefficient for α1AT at 280 nm of 5.3 (42, 43). Values for the α1AT 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 α1AT from lavage fluid was determined in a titration assay as previously described (44). Levels of α1AT 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 α1AT from lavage fluid and plasma. α1AT 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 α1AT 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-α1AT 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 α1AT 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 α1AT peak was collected and then circulated for 12 h over a "negative selection" affinity column containing antibodies against human whole serum, albumin, prealbumin, α2-macroglobulin, chymotrypsin, orosomucoid, IgG, IgA, IgM, C-1 esterase, and antithrombin III (all from Accurate Chemical and Scientific Corp.) (45). Purified α1AT collected from this column was concentrated by pressure filtration (YM-10). The final concentration of purified α1AT 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 α1AT for human neutrophil elastase. The association rate constant (k assoc) of α1AT 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 α1AT 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 α1AT (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 α1AT 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 α1AT for human neutrophil elastase. The relevance of the association rate constant of an inhibitor such as α1AT 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 α1AT inhibition time" is an estimate of the time required for five half-lives of the reaction of the α1AT with a burden of elastase based on the amount of α1AT present in the biologic fluid of interest. Using the formulation of Bieth (48, 49), the in vivo lung α1AT inhibition time was quantified as: 5/[(k assoc) $\times$ (concen-

tration of functional α1AT in ELF)]. For each individual, the in vivo lung α1AT inhibition time was quantified using that individual's values for ELF α1AT k assoc and ELF functional α1AT concentrations. These individual in vivo inhibition times were used for the calculation of the group times for smokers and nonsmokers.

Results

α1-Antitrypsin levels. The α1AT 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 μM, smokers 4.0±0.2 μM, $P > 0.5$). In addition, no difference was observed in the amount of α1AT in lavage fluid when referenced to the amount of albumin present (nonsmokers 52±6 μg α1AT/mg albumin, smokers 49±7 μg/mg albumin, $P > 0.5$). Thus on the basis of the amounts of total α1AT present in plasma or in ELF, normal nonsmokers and smokers cannot be distinguished. Evaluation of epithelial lining fluid α1AT activity using the time-independent assay for activity revealed that nonsmokers' α1AT was 78.4±3.7% active while smokers' ELF was 62.3±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 α1AT activity of nonsmokers' and smokers' ELF α1AT, 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 α1AT for neutrophil elastase. There was no difference in the ability of α1AT in the plasma of nonsmokers and smokers to inhibit neutrophil elastase in a time-dependent fashion. In this regard, the k assoc of plasma α1AT for neutrophil elastase for nonsmokers was $9.3\pm0.7 \times 10^6$ $M^{-1}s^{-1}$ and for smokers was $9.5\pm0.5 \times 10^6$ $M^{-1}s^{-1}$, $P > 0.5$). Thus, when in the vascular compartment, the α1AT molecules in nonsmokers has a similar potential to inhibit neutrophil elastase as do the plasma α1AT molecules in cigarette smokers.

In marked contrast to the vascular compartment, comparison of the k assoc for neutrophil elastase of α1AT purified from lavage fluid was significantly lower for smokers ($6.5\pm0.1 \times 10^6$ $M^{-1}s^{-1}$) than nonsmokers ($8.1\pm0.1 \times 10^6$ $M^{-1}s^{-1}$, $P < 0.01$) (Fig. 1). Thus, for cigarette smokers, on the average, the α1AT 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 α1AT molecules at the same site in nonsmokers.

Estimates of in vivo relevance. The in vivo relevance of the reduced k assoc of smoker lung α1AT for neutrophil elastase is vividly conceptualized by estimating the in vivo α1AT lung inhibition time for neutrophil elastase (Fig. 2). In this regard, for the levels of functional α1AT found in ELF for each individual, the k assoc of smoker lung α1AT translates into an in vivo inhibition time of 0.34±0.01 s, while the k assoc of nonsmoker lung α1AT 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 α1AT molecules in the smoker ELF would take twice as long to effectively inhibit the elastase burden.

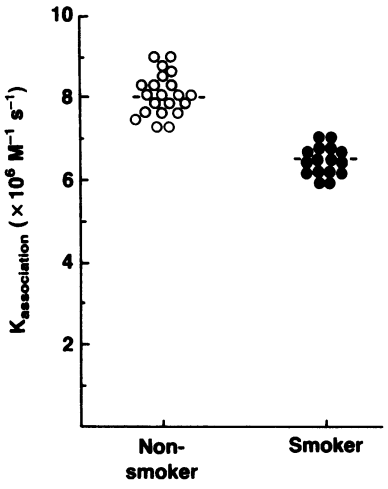

Figure 1. Comparison of the association rate constant of nonsmoker and smoker lung α1-antitrypsin for neutrophil elastase. α1AT purified from lavage fluid was incubated with neutrophil elastase for varying indicated times and the k association of the α1AT for neutrophil elastase quantified. Shown are data from 21 lung α1AT samples from 12 nonsmokers (○) and 16 samples of lung α1AT 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).

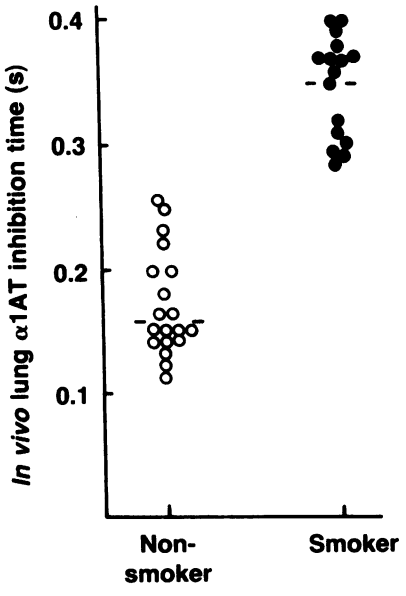

Figure 2. In vivo lung α1-antitrypsin inhibition time for neutrophil elastase. The in vivo inhibition time is an estimate of the time required to inhibit neutrophil elastase relevant to the concentration of the α1AT present (see Methods). Data are shown for 19 samples from 11 nonsmokers (○) and 16 samples 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 α1AT (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 α1AT 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 α1AT 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 α2-macroglobulin in addition to α1AT, it is unclear whether the effect that was seen was due to reduced neutrophil elastase inhibitory capacity of α1AT or to other factors present in lavage fluid. Capitalizing on the development of methods to purify the small quantities of α1AT 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 α1AT, we have been able to ask whether the α1AT molecules in the lungs of smokers are similar or different from the α1AT 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 α1AT 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 α1AT can be appreciated by comparison of the risk of developing emphysema among cigarette smokers with normal α1AT levels to the emphysema risk of individuals with the α1AT phenotype MZ, who have serum α1AT levels as low as 18 μM. 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 α1AT. 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 α1AT 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 α1AT 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 Met^{358} residue at the active inhibitory site of the α1AT 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 α1AT is exposed to cigarette smoke (24, 27, 29) or to activated alveolar macrophages or neutrophils (58–61), the active inhibitory site of the α1AT 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 α1AT present in the lower respiratory tract (30). Fifth, when the active inhibitory site of α1AT is oxidized, the association rate constant for neutrophil elastase is reduced (47). Sixth, direct analysis of the methionine residues in α1AT 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 α1AT represents a mixture of oxidized and normal nonoxidized α1AT 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 α1AT from plasma into the alveolar space may influence the status of α1AT within the lower respiratory tract. These variables will have the effect that not all α1AT 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 α1AT molecular population composed of a mixture of oxidized and nonoxidized α1AT.

Since the α1AT 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 α1AT to protect lung macromolecules such as elastin or collagen is not feasible. It has been shown, however, that oxidized α1AT 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 α1AT 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 α1AT molecules in the smoker lung is not an "all or none" phenomenon; due to the complex interplay of ventilation, oxidants, and antioxidants, and α1AT turnover, lung α1AT is not in a completely oxidized form. Finally, the fact that the relative impotence of the α1AT 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.

References

1. Snider, G. L., J. Kleinerman, W. M. Thurlbeck, and Z. H. Bengali. 1985. The definition of emphysema. Report of a National Heart, Lung, and Blood Institute, Division of Lung Diseases Workshop. *Am. Rev. Respir. Dis.* 132:182–185.

2. Thurlbeck, W. M., J. A. Henderson, R. G. Fraser, and D. V. Bates. 1970. Chronic obstructive lung disease. A comparison between clinical, roentgenologic, functional and morphologic criteria in chronic bronchitis, emphysema, asthma and bronchiectasis. *Medicine (Baltimore).* 49:81–145.

3. Hugh-Jones, P., and W. Whimster. 1978. The etiology and management of disabling emphysema. *Am. Rev. Respir. Dis.* 117:343–377.

4. Smoking and Health; Report of the Advisory Committee to the Surgeon General of the Public Health Service. U.S. Dept of Health, Education, and Welfare. 1984. Washington, D.C. p. 14–57.

5. Auerbach, O., E. C. Hammond, L. Garfinkel, and C. Benante. 1972. Relation of smoking and age to emphysema. Whole-lung section study. *N. Engl. J. Med.* 286:853–857.

6. Janoff, A. 1985. Elastases and emphysema. Current assessment of the protease-antiprotease hypothesis. *Am. Rev. Respir. Dis.* 132:417–433.

7. Gadek, J. E., G. A. Fells, R. L. Zimmerman, S. I. Rennard, and R. G. Crystal. 1981. Antielastases of the human alveolar structures. Implications for the protease-antiprotease theory of emphysema. *J. Clin. Invest.* 68:889–898.

8. Stone, P. J. 1983. The elastase-antielastase hypothesis of the pathogenesis of emphysema. *Clin. Chest Med.* 4:405–412.

9. Laurell, C. B., and S. Eriksson. 1963. The electrophoretic α1-globulin pattern of serum in α1-antitrypsin deficiency. *Scand. J. Clin. Lab. Invest.* 15:132–140.

10. Gross, P., E. A. Pfitzer, E. Tolker, M. A. Babyak, and M. Kaschak. 1965. Experimental emphysema: its production with papain in normal and silicotic rats. *Arch. Environ. Health.* 11:50–58.

11. Gadek, J. E., and R. G. Crystal. 1982. α1-antitrypsin deficiency. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, and M. S. Brown, editors. McGraw-Hill Inc., New York. 1450–1467.

12. Carrell, R. W., J.-O. Jeppsson, C.-B. Laurell, S. O. Brennan, M. C. Owen, L. Vaughan, and D. R. Boswell. 1982. Structure and variation of human α1-antitrypsin. *Nature (Lond.).* 298:329–334.

13. Janoff, A., R. White, H. Carp, S. Harel, R. Dearing, and D. Lee. 1979. Lung injury induced by leukocytic proteases. *Am. J. Pathol.* 97:111–129.

14. Bieth, J. G. 1986. Elastases: catalytic and biological properties. *In* Regulation of Matrix Accumulation. R. Mechan, editor. Academic Press, New York. 217–320.

15. Travis, J., and G. Salvesen. 1983. Human plasma proteinase inhibitors. *Annu. Rev. Biochem.* 52:655–709.

16. Perlmutter, D. H., F. S. Cole, P. Kilbridge, T. H. Rossing, and H. R. Colten. 1985. Expression of the α1-proteinase inhibitor gene in human monocytes and macrophages. *Proc. Natl. Acad. Sci. USA.* 82:795–799.

17. Mornex, J.-F., A. Chytil-Weir, Y. Martinet, M. Courtney, J.-P. LeCocq, and R. G. Crystal. 1986. Expression of the alpha 1-antitrypsin gene in mononuclear phagocytes of normal and alpha 1-antitrypsin deficient individuals. *J. Clin. Invest.* 77:1952–1961.

18. Kueppers, F., and L. F. Black. 1974. α1-antitrypsin and its deficiency. *Am. Rev. Respir. Dis.* 110:176–194.

19. Morse, J. O. 1978. Alpha 1-antitrypsin deficiency. *N. Engl. J. Med.* 299:1045–1048 and 1099–1105.

20. Hunninghake, G. W., and R. G. Crystal. 1983. Cigarette smoking and lung destruction: accumulation of neutrophils in the lungs of cigarette smokers. *Am. Rev. Respir. Dis.* 128:833–838.

21. Ludwig, P. W., B. A. Schwartz, J. R. Hoidal, and D. E. Niewoehner. 1985. Cigarette smoking causes accumulation of polymorphonuclear leukocytes in alveolar septum. *Am. Rev. Respir. Dis.* 131:828–830.

22. Olsen, G. N., J. O. Harris, J. R. Castle, R. H. Waldman, and H. J. Karmgard. 1975. Alpha-1-antitrypsin content in the serum, alveolar macrophages, and alveolar lavage fluid of smoking and nonsmoking normal subjects. *J. Clin. Invest.* 55:427–430.

23. Gadek, J. E., G. A. Fells, and R. G. Crystal. 1979. Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. *Science (Wash. DC).* 206:1315–1316.

24. Janoff, A., and H. Carp. 1977. Possible mechanisms of emphysema in smokers: cigarette smoke condensate suppresses protease inhibition in vitro. *Am. Rev. Respir. Dis.* 116:65–72.

25. Janoff, A., H. Carp, and D. K. Lee. 1979. Cigarette smoke inhalation decreases α1-antitrypsin activity in rat lung. *Science (Wash. DC).* 206:1313–1314.

26. Johnson, D., and J. Travis. 1979. The oxidative inactivation of human α-1-proteinase inhibitor: further evidence for methionine at the active center. *J. Biol. Chem.* 254:4022–4026.

27. Pryor, W. A., M. M. Dooley, and D. F. Church. 1986. The mechanisms of the inactivation of human alpha-1-proteinase inhibitor by gas-phase cigarette smoke. *Adv. Free Radical Biol. in Med.* 2:161–188.

28. Carp, H., and A. Janoff. 1980. Potential mediator of inflammation. Phagocyte-derived oxidants suppress the elastase-inhibitory capacity of alpha 1-proteinase inhibitor in vitro. *J. Clin. Invest.* 66:987–995.

29. Cohen, A. B., and H. L. James. 1982. Reduction of the elastase inhibitory capacity of alpha 1-antitrypsin by peroxides in cigarette smoke: an analysis of brands and filters. *Am. Rev. Respir. Dis.* 126:25–30.

30. Hubbard, R., G. Fells, A. Cantin, F. Ogushi, M. Courtney, and R. G. Crystal. 1986. Recombinant DNA produced val^{358} α1-antitrypsin more effectively resists oxidative inactivation by alveolar macrophages of cigarette smokers than plasma-derived M1M1 α1-antitrypsin or met^{358} α1-antitrypsin. *Am. Rev. Respir. Dis.* 133:A218.

31. Carp, H., F. Miller, J. R. Hoidal, and A. Janoff. 1982. Potential mechanism of emphysema: α1-proteinase inhibitor recovered from lungs of cigarette smokers contains oxidized methionine and has decreased elastase inhibitory capacity. *Proc. Natl. Acad. Sci. USA.* 79:2041–2045.

32. Abboud, R. T., T. Fera, A. Richter, M. Z. Tabona, and S. Johal. 1985. Acute effect of smoking on the functional activity of alpha 1-protease inhibitor in bronchoalveolar lavage fluid. *Am. Rev. Respir. Dis.* 131:79–85.

33. El-Yamani, J., A. Hayem, J. J. Lafitte, B. Gressier, and J. Mizon. 1986. Functional and immunoreactive α1-proteinase inhibitor in bronchoalveolar lavages: methodological studies. *Bull. Eur. Physiopathol. Respir.* 22:359–363.

34. Stone, P. J., J. D. Calore, S. E. McGowan, J. Bernardo, G. L. Snider, and C. Franzblau. 1983. Functional α1-protease inhibitor in the lower respiratory tract of cigarette smokers is not decreased. *Science (Wash. DC).* 221:1187–1189.

35. Boudier, C., A. Pelletier, G. Pauli, and J. G. Bieth. 1983. The functional activity of α1-proteinase inhibitor in bronchoalveolar lavage fluids from healthy human smokers and non-smokers. *Clin. Chim. Acta.* 132:309–315.

36. Jochum, M., A. Pelletier, C. Boudier, G. Pauli, and J. G. Bieth. 1985. The concentration of leukocyte elastase-α1-proteinase inhibitor complex in bronchoalveolar lavage fluids from healthy human subjects. *Am. Rev. Respir. Dis.* 132:913–914.

37. Wewers, M. D., D. J. Herzyk, and J. E. Gadek. 1989. Comparison of smoker and nonsmoker lavage fluid for the rate of association with neutrophil elastase. *Am. J. Respir. Cell. Mol. Biol.* 1:423–429.

38. Cox, W., A. M. Johnson, and M. K. Fagerhol. 1980. Report of nomenclature meeting for α1-antitrypsin. *Hum. Genet.* 53:429–433.

39. Saltini, C., A. J. Hance, V. J. Ferrans, F. Basset, P. B. Bitterman, and R. G. Crystal. 1984. Accurate quantification of cells recovered by bronchoalveolar lavage. *Am. Rev. Respir. Dis.* 130:650–658.

40. Rennard, S. I., G. Basset, D. Lecossier, K. M. O'Donnell, P. Pinkston, P. G. Martin, and R. G. Crystal. 1986. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. *J. Appl. Physiol.* 60:532–538.

41. Rennard, S. I., R. Berg, G. R. Martin, J. M. Foidart, and P. G. Robey. 1980. Enzyme-linked immunoassay (ELISA) for connective tissue components. *Anal. Biochem.* 104:205–214.

42. Pannell, R., D. Johnson, and J. Travis. 1974. Isolation and properties of human plasma α-1-proteinase inhibitor. *Biochemistry.* 13:5439–5445.

43. Jeppsson, J.-O., C.-B. Laurell, and M. Fagerhol. 1978. Properties of isolated human α1-antitrypsins of Pi types M, S and Z. *Eur. J. Biochem.* 83:143–153.

44. Straus, S. D., G. A. Fells, M. D. Wewers, M. Courtney, L.-H. Tessier, P. Tolstoshev, J.-P. LeCocq, and R. G. Crystal. 1985. Evaluation of recombinant DNA-directed E. coli produced α1-antitrypsin as an anti-neutrophil elastase for potential use as replacement therapy of α1-antitrypsin deficiency. *Biochem. Biophys. Res. Commun.* 130:1177–1184.

45. Laurell, C.-B., J. Pierce, U. Persson, and E. Thulin. 1975. Purification of α1-antitrypsin from plasma through thiol-disulfide interchange. *Eur. J. Biochem.* 57:107–113.

46. Sugiura, M., S. Hayakawa, T. Adachi, Y. Ito, K. Hirano, and S. Sawaki. 1981. A simple one-step purification of human α1-proteinase inhibitor by immunoadsorbent column chromatography. *J. Biochem. Biophys. Meth.* 5:243–249.

47. Beatty, K., J. Bieth, and J. Travis. Kinetics of association of serine proteinases with native and oxidized α-1-proteinase inhibitor and α-1-antichymotrypsin. 1980. *J. Biol. Chem.* 255:3931–3934.

48. Bieth, J. G. 1984. In vivo significance of kinetic constants of protein proteinase inhibitors. *Biochem. Med.* 32:387–397.

49. Bieth, J. G. 1980. Pathophysiological interpretation of kinetic constants of protease inhibitors. *Bull. Eur. Physiopathol. Respir.* 16(Suppl.):183–195.

50. Hance, A. J., and R. G. Crystal. 1975. The connective tissue of lung. *Am. Rev. Respir. Dis.* 112:657–711.

51. Karlinsky, J. B., and G. L. Snider. 1978. Animal models of emphysema. *Am. Rev. Respir. Dis.* 117:1109–1133.

52. Damiano, V. V., A. Tsang, U. Kucich, W. R. Abrams, J. Rosenbloom, P. Kimbel, M. Fallahnejad, and G. Weinbaum. 1986. Immunolocalization of elastase in human emphysematous lungs. *J. Clin. Invest.* 78:482–493.

53. Janoff, A., L. Raju, and R. Dearing. 1983. Levels of elastase activity in bronchoalveolar lavage fluids of healthy smokers and nonsmokers. *Am. Rev. Respir. Dis.* 127:540–544.

54. Morse, J. D., M. D. Lebowitz, M. D. Knudson, and B. Burrows. 1975. A community study of the relationship of alpha 1-antitrypsin levels to obstructive lung diseases. *N. Engl. J. Med.* 292:278–281.

55. Bruce, R. M., B. H. Cohen, E. L. Diamond, R. J. Fallat, R. J. Knudson, M. D. Lebowitz, C. Mittman, C. D. Patterson, and M. S. Tockman. 1984. Collaborative study to assess risk of lung disease in PiMZ phenotype subjects. *Am. Rev. Respir. Dis.* 130:386–390.

56. Hoidal, J. R., R. B. Fox, P. A. LeMarbe, R. Perri, and J. E. Repine. 1975. Altered oxidative metabolic responses in vitro of alveolar macrophages from asymptomatic cigarette smokers. *Am. Rev. Respir. Dis.* 123:85–89.

57. Richter, A. M., R. T. Abboud, S. S. Johal, and T. A. Fera. 1986. Acute effect of smoking on superoxide production by pulmonary alveolar macrophages. *Lung.* 164:233–242.

58. Carp, H., and A. Janoff. 1979. In vitro suppression of serum elastase-inhibitory capacity by reactive oxygen species generated by phagocytosing polymorphonuclear leukocytes. *J. Clin. Invest.* 63:793–797.

59. Janoff, A., C. George-Nascimento, and S. Rosenberg. 1986. A genetically engineered, mutant human alpha-1-proteinase inhibitor is more resistant than the normal inhibitor to oxidative inactivation by chemicals, enzymes, cells, and cigarette smoke. *Am. Rev. Respir. Dis.* 133:353–356.

60. Zaslow, M. C., R. A. Clark, P. J. Stone, J. D. Calore, G. L. Snider, and C. Franzblau. 1983. Human neutrophil elastase does not bind to alpha 1-protease inhibitor that has been exposed to activated human neutrophils. *Am. Rev. Respir. Dis.* 128:434–439.

61. Clark, R. A., P. J. Stone, A. El Hag, J. D. Calore, and C. Franzblau. 1981. Myeloperoxidase-catalyzed inactivation of α1-protease inhibitor by human neutrophils. *J. Biol. Chem.* 256:3348–3353.

62. Niewoehner, D. D., J. Kleinerman, and D. B. Rice. 1974. Pathologic changes in the peripheral airways of young cigarette smokers. *N. Engl. J. Med.* 291:755–758.

63. Beatty, K., N. Matheson, and J. Travis. 1984. Kinetic and chemical evidence for the inability of oxidized α1-proteinase inhibitor to protect lung elastin from elastolytic degradation. *Hoppe-Seyler's Z. Physiol. Chem.* 365:S731–736.