Oxidants Spontaneously Released by Alveolar Macrophages of Cigarette Smokers Can Inactivate the Active Site of α1-Antitrypsin, Rendering It Ineffective as an Inhibitor of Neutrophil Elastase

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Abstract

Current concepts relating to the pathogenesis of emphysema associated with cigarette smoking is that an imbalance exists within the lower respiratory tract between neutrophil elastase and the local anti-neutrophil elastase screen, enabling uninhibited neutrophil elastase to destroy the alveolar structures over time. The possible role of alveolar macrophages in contributing to this imbalance was investigated by evaluating the ability of cigarette smokers' alveolar macrophages to inactivate alpha 1-antitrypsin (α1AT), the major anti-neutrophil elastase of the human lower respiratory tract. In vitro, alveolar macrophages of smokers spontaneously released 2.5-fold more superoxide anion and eightfold more H_2O_2 than macrophages of nonsmokers ($P < 0.01$, both comparisons). Using a model system that reproduced the relative amounts of alveolar macrophages and α1AT found in the epithelial lining fluid of the lower respiratory tract, we observed that smokers' macrophages caused a 60±5% reduction in the ability of α1AT to inhibit neutrophil elastase. In marked contrast, under the same conditions, nonsmokers' macrophages had no effect upon the anti-neutrophil elastase function of α1AT. Addition of superoxide dismutase, catalase, mannitol, and methionine prevented inactivation of α1AT by smokers' macrophages, implying that the release of oxidants mediated the inactivation of α1AT. In addition, by utilizing a recombinant DNA produced modified form of α1AT containing an active site substitution ($met^{358} \rightarrow val$), the inactivation of α1AT by smokers' alveolar macrophages was prevented, suggesting that the smokers' macrophages inactivate α1AT by oxidizing the active site of the α1AT molecule. These results suggest that in cigarette smokers, the alveolar macrophage can modulate the activity of α1AT as an inhibitor of neutrophil elastase and thus play a role in the pathogenesis of emphysema associated with cigarette smoking.

Introduction

Alpha 1-antitrypsin (α1AT),[1] is a 52-kD glycoprotein that serves as the major inhibitor of neutrophil elastase, a potent serine protease that is capable of attacking most protein components of the extracellular matrix (1–3). The critical importance of α1AT as an inhibitor of neutrophil elastase is highlighted by α1AT deficiency, a hereditary disorder associated with plasma α1AT levels < 35% of normal and the development of emphysema in the third to fourth decades (4–7). In this context, the pathogenesis of the emphysema associated with α1AT deficiency is conceptualized as an imbalance between α1AT and neutrophil elastase in the alveolar structures such that there is insufficient α1AT to provide a protective screen against the burden of neutrophils, and hence neutrophil elastase, in the local milieu (4, 8, 9).

The same concepts have been extended to conceptualize the pathogenesis of the emphysema associated with cigarette smoking (10–15). Despite the fact that most of these individuals have normal levels of α1AT in blood and lung (16, 17), the knowledge that α1AT can be rendered impotent by oxidation has led to the concept that cigarette smoking may lead to an inactivation of α1AT in the lung, leaving the lower respiratory tract without its normal anti-neutrophil elastase defenses (1, 12–15, 18). This concept is supported by in vitro studies showing that cigarette smoke will inactivate α1AT by oxidative mechanisms (12, 19–21) and that the α1AT recovered from the epithelial surface of the lower respiratory tract of cigarette smokers has a reduced association rate constant for neutrophil elastase (22). Furthermore, the α1AT recovered from the lung of cigarette smokers has been observed to have a decreased ability to inhibit porcine pancreatic elastase compared with the lung α1AT of nonsmokers (13, 23, 24), although this observation has been disputed (25, 26).

Although it is compelling to invoke cigarette smoke, with its high concentration of oxidants (20), as a source of the oxidant burden that potentially could inactivate α1AT in the lower respiratory tract, another potential source of oxidants in the lower respiratory tract of cigarette smokers are alveolar macrophages. In this regard: (*a*) when activated, alveolar macrophages are capable of releasing oxidants, including superoxide anion, hydrogen peroxide, and hydroxyl radical (27, 28); (*b*) the number of alveolar macrophages in the lower respiratory tract of cigarette smokers is increased severalfold (17, 29, 30); (*c*) when activated in vitro, alveolar macrophages can release sufficient oxidants to render α1AT an ineffective inhibitor of neutrophil elastase (31, 32); and (*d*) alveolar macrophages recovered from the lungs of cigarette smokers are spontaneously releasing oxidants (29, 33). In the context of these observations it is reasonable to hypothesize that alveolar macrophages in the lower respiratory tract of cigarette smokers

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Received for publication 20 March 1987 and in revised form 30 June 1987.

1. *Abbreviations used in this paper:* α1AT, alpha 1-antitrypsin; ELF, epithelial lining fluid; MeO-SAAPV-NA, methoxy-succinyl-alanyl-alanyl-prolyl-valine nitroanilide; O_2^-, superoxide anion; SOD, superoxide dismutase.

may be spontaneously releasing sufficient oxidants to inactivate α1AT in the local milieu.

To evaluate this hypothesis, we recovered alveolar macrophages from the lower respiratory tract of nonsmokers and smokers, and evaluated the ability of these cells to modulate the activity of α1AT as an inhibitor of neutrophil elastase. In addition, by utilizing a recombinant DNA-produced modified form of α1AT containing an active site substitution (met^{358} → val) rendering it resistant to oxidation but still active as an inhibitor of neutrophil elastase, we have demonstrated that the spontaneous inactivation of α1AT by macrophages of cigarette smokers occurs, at least in part, by oxidation of the active site of the α1AT.

Methods

Study population. The study population consisted of 5 healthy nonsmoking individuals (three male, two female; age 31±7 yr) and 10 healthy smoking individuals (six male, four female; age 32±6 yr) with an average cigarette smoking history of 27±11 pack years. (Data is presented as mean±SE of the mean; all statistical comparisons were carried out using the two-tailed Student's *t* test.) All individuals were free of lung disease as determined by the combined criteria of having normal histories, physical examinations, chest x rays, and lung function tests (34).

Lower respiratory tract inflammatory cells. Inflammatory cells were recovered from the lower respiratory tract of nonsmokers and smokers by bronchoalveolar lavage as previously described (35). Alveolar macrophages were purified by adherence (1 h, 37°) at 5×10^5 cells in 1 ml in 24-well tissue culture plates (Falcon Labware, Oxnard, CA) in RPMI medium (Gibco, Grand Island, NY) containing 10% calf serum (Whitaker M. A. Bioproducts, Walkersville, MD). In all cases the alveolar macrophages were > 95% pure and > 90% viable (as assessed by trypan blue). The volume of epithelial lining fluid (ELF) recovered, quantified using the urea method (36), was 2.0±1.0 ml for nonsmokers and 2.7±1.1 ml for smokers ($P > 0.2$). Concentrations of α1AT in ELF were quantified using an enzyme-linked immunoassay (in quadruplicate) with a standard α1AT that had been quantified by amino acid analysis (37).

The spontaneous release of superoxide anion (O_2^-) and H_2O_2 by the purified alveolar macrophages of the nonsmokers and smokers was quantified in vitro (38, 39). Briefly, for O_2^-, the alveolar macrophages (5×10^5/well) in 24-well plates were incubated in 0.5 ml of Hanks' balanced salt solution (HBSS; Gibco) containing 80 nm ferricytochrome *c* (Type III, Sigma Chemical Co., St. Louis, MO). After incubation for 30 min at 37°, the amount of O_2^- released into the medium was quantified at 550 nm. For the measurement of the spontaneous release of H_2O_2, HBSS containing 0.28 mM phenol red, 8.5 U/ml horseradish peroxidase (Type II, Sigma Chemical Co.) was added and the macrophages incubated for 30 min at 37°. The amount of H_2O_2 in the supernatant was then determined by the addition of 10 μl of 1 N NaOH and the absorbance read at 610 nm. The concentration of H_2O_2 was based on a standard curve using various concentrations of reagent grade H_2O_2.

Source of α1AT. Normal human α1AT was purified from the plasma of an individual homozygous for the M1(val^{213}) form of α1AT as identified by the combined criteria of isoelectric focusing, serum α1AT levels, family studies, and BstEII restriction endonuclease mapping of genomic DNA (40, 41). Purification of α1AT from plasma was accomplished by positive selection affinity chromatography (42). The purified α1AT was concentrated by pressure filtration (YM-10 membrane, Amicon Corp., Danvers, MA) and stored in aliquots in liquid nitrogen vapor until use. The final preparation was > 95% pure as assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Recombinant α1AT was produced in *Escherichia coli* as previously described (43, 44), using an α1AT complementary DNA (cDNA) obtained from translation of human liver RNA. Competent TGE900 *E. coli* cells were transformed with an expression plasmid containing the α1AT cDNA and the recombinant α1AT purified from bacterial lysates. Two forms of recombinant α1AT were produced. The normal form (met^{358} rα1AT) has the identical sequence of the M1(val^{213}) form of human α1AT present in plasma except for an additional NH_2-terminal methionine and the absence of carbohydrates. It is designated met^{358} rα1AT to indicate that it contains a methionine in the active inhibitory site, as does the normal M1(val^{213}) protein. The val^{358} rα1AT form was identical to the met^{358} rα1AT except for the substitution of val^{358} at the active site. The val^{358} rα1AT has been shown to be similar to the met^{358} rα1AT in terms of its ability to inhibit human neutrophil elastase, but the val^{358} rα1AT is much more resistant to oxidation (44–48).

Evaluation of ability of the α1AT preparations to inhibit neutrophil elastase. The ability of α1AT to inhibit neutrophil elastase was evaluated using two assays: (*a*) the time-dependent inhibition of equimolar concentration of neutrophil elastase and α1AT; and (*b*) the time-independent inhibition of neutrophil elastase by increasing concentrations of α1AT. Both assays were carried out using a neutrophil elastase standard for which the activity had been determined (37).

The time-dependent inhibition of purified α1AT with neutrophil elastase was carried out by the method of Beatty et al. (11) with minor modifications described by Straus et al. (37). In brief, the α1AT was titrated using neutrophil elastase to determine the percentage of active α1AT in the preparation. Equimolar amounts of elastase and active α1AT (1 nM each) were then reacted at 23° in a 1-ml reaction volume containing 100 mM Hepes, pH 7.5, 0.5 M NaCl, and 0.1% Brij-35. Residual elastase activity at various times was determined by terminating the reaction by the addition of the specific neutrophil elastase substrate methoxy-succinyl-alanyl-alanyl-prolyl-valine nitroanilide (MeO-SAAPV-NA; 1 mM; Sigma Chemical Co.) and the residual elastase activity measured at 410 nm as a change in optical density per minute using a DU-7 spectrophotometer (Beckman Instruments, Fullerton, CA). At each time point the percent inhibition of elastase was then quantified.

Time-independent inhibition of neutrophil elastase by α1AT was determined by incubating a fixed amount (2 nM) of active neutrophil elastase with various concentrations (0–8 nM) of α1AT at 23° for 2 h, a time at which the time-dependent assays demonstrated were at least twofold longer than necessary for reaction of neutrophil elastase with the α1AT (11, 49). After the incubation, the neutrophil elastase–specific substrate MeO-SAAPV-NA was added and percent residual elastase activity was determined as previously described.

Evaluation of ability of smoker alveolar macrophages to spontaneously inactivate α1AT as an inhibitor of elastase. The ability of smokers' alveolar macrophages to spontaneously inactivate α1AT was evaluated using an in vitro assay that mimicked the numbers of macrophages and concentrations of α1AT in the epithelial lining fluid of the lower respiratory tract. To accomplish this, alveolar macrophages recovered from the lower respiratory tract of smokers and nonsmokers were exposed in vitro to purified plasma α1AT (type M1[val^{213}], see previous section) in a chamber designed to permit only low-molecular weight products of the macrophages to reach the α1AT. The chamber was divided in two sections, a lower α1AT-containing section and an upper macrophage chamber. The total volume of the chamber was 1,400 μl (upper section, 1,000 μl + lower section, 400 μl). Separating the chambers was a dialysis membrane (Union Carbide Corp., Danbury, CT) with a molecular weight cutoff of 12,000–14,000 kD. Alveolar macrophages (2×10^6) from a nonsmoker or a smoker were placed in HBSS containing 0.1% glucose in the upper chamber and α1AT (1 μM in HBSS containing 0.1% glucose) was placed in the lower chamber. The numbers of alveolar macrophages and the amounts of α1AT used were always in the ratio of 4.65×10^9 macrophages/μmol α1AT. This value was based on the average ratio of macrophages to α1AT recovered in the ELF of nonsmokers, and is approximately threefold less than that observed in smokers, i.e., the design was based

on that found in normals and was biased against the increased ratio of macrophages to α1AT present in the lower respiratory tract of smokers. Control samples contained α1AT in the lower chamber but no added alveolar macrophages. After the chambers were incubated for 18 h at 37°, α1AT was retrieved from the lower portion of the chamber and then evaluated for its ability to inhibit neutrophil elastase, as previously described.

To demonstrate that low-molecular weight mediators of smoker alveolar macrophages that inactivated the α1AT were oxidants, parallel incubations were carried out using alveolar macrophages from smokers and purified plasma α1AT but with the addition of antioxidants to the chambers. Superoxide dismutase (SOD; 400 U/ml, Type III, Sigma Chemical Co.), catalase (1,000 U/ml, bovine liver, Sigma Chemical Co.), or a combination of both antioxidants were added to both the macrophage and α1AT portions of the chambers and the chambers incubated as previously described. In selected experiments the hydroxyl radical ($OH^{\cdot}$) scavenger mannitol (1 mM) or methionine (1 mM) (which inactivates HOCl) was added. After incubation, the α1AT was retrieved and its ability to inhibit porcine pancreatic elastase (Calbiochem-Behring Corp., La Jolla, CA) was measured. Pancreatic elastase was specifically used in this experiment because when the active site met^{358} residue of α1AT is oxidized, the α1AT molecule completely loses its ability to inhibit porcine pancreatic elastase. (In contrast, it is still able to inhibit neutrophil elastase, albeit with a markedly reduced association rate constant [11]). Thus, although porcine pancreatic elastase is not the normal physiologic target of human α1AT in vivo, it offers a convenient method to analyze the protective effect of antioxidants. The reaction between the α1AT recovered from the chambers and the porcine pancreatic elastase was carried out in a 1-ml reaction volume containing 100 mM Tris-HCl, pH 8.3, 0.5 M NaCl, 0.1% Brij-35, and 8 nM porcine pancreatic elastase (11). Varying concentrations of α1AT were added and the reaction mixture incubated for 3 h at 23°. After incubation, the porcine pancreatic elastase-specific substrate (*n*-succinyl-alanyl-alanyl-alanyl-NA, 1 mM, Calbiochem-Behring Corp.) was added to the incubation. The residual pancreatic elastase activity was then quantified by measuring the change in optical density at 410 nm, and percent inhibition of elastase was calculated as previously described.

To directly demonstrate the involvement of the α1AT met^{358} residue as the target for the smoker alveolar macrophage–released oxidants as the mechanism for inactivating the α1AT, we carried out identical experiments as described for the smoker macrophages and the plasma α1AT, but recombinant forms of α1AT, met^{358} rα1AT, and val^{358} rα1AT were substituted for the plasma α1AT. Briefly, smokers' alveolar macrophages were added to the upper portion of a chamber across a dialysis membrane from either met^{358} α1AT or val^{358} α1AT in the same ratios used previously. After incubation for 18 h at 37° the recombinant α1AT was retrieved and evaluated for its ability to inhibit neutrophil elastase as previously described.

Results

Relationship between alveolar macrophages and α1AT on the alveolar epithelial surface. Evaluation of lavage fluid demonstrated that, relative to the amounts of α1AT present, the environment of the epithelial lining fluid of the lower respiratory tract of nonsmokers and cigarette smokers were substantially different both in number of alveolar macrophages and amount of oxidants released per hour. In this regard, nonsmokers had an average of $21.4\pm14.2 \times 10^3$ alveolar macrophages per μl of lower respiratory tract epithelial lining fluid, whereas the smokers had $43.2\pm26.3 \times 10^3/\mu l$ ELF ($P < 0.01$). However, the concentration of α1AT in ELF was similar in the two groups (nonsmokers, 4.5±0.8 μM; smokers, 3.21±1.0 μM; $P > 0.1$). The ELF of smokers contained nearly threefold more alveolar macrophages per mole of α1AT than did nonsmokers (Fig. 1 *A*; $P < 0.01$). Not only did the smokers have relatively more alveolar macrophages, but the macrophages were activated and spontaneously releasing more oxidants than the macrophages of nonsmokers. In this context nonsmoker alveolar macrophages were releasing 18.2±8.3 nmol $O_2^+/10^6$ alveolar macrophages per h and 1.6±0.6 nmol $H_2O_2/10^6$ macrophages per h, whereas smoker macrophages were releasing 42.4±13.3 nmol $O_2^+/10^6$ macrophages per h and 13.1±4.2 nmol $H_2O_2/10^6$ macrophages per h (nonsmokers vs. smokers, $P < 0.01$ for O_2^+ and H_2O_2, respectively). When considered in terms of amounts of α1AT present in ELF, it was apparent that smokers' alveolar macrophages present a burden of oxidants markedly greater than do nonsmoker macrophages. For example, nonsmoker macrophages spontaneously released 84±12 mol/h^{-1} O_2^+ per mol α1AT in ELF, whereas smoker macrophages released 570±120 mol/h^{-1} O_2^+ per mol α1AT ($P < 0.005$). The burden of H_2O_2 released by the alveolar macrophages was even greater. Smoker alveolar macrophages released 28-fold more H_2O_2 per mol of α1AT in ELF than did nonsmoker alveolar macrophages (Fig. 1 *B*, $P < 0.001$). Thus, on the average, the oxidant burden from alveolar macrophages faced by a molecule of α1AT within the smokers' alveolus is far greater than that within the nonsmokers' alveolus, and consequently the likelihood of oxidative alteration of the properties of the α1AT molecule is correspondingly much greater within the smokers' lung than in the lung of the nonsmoker.

Analysis of ability of nonsmoker and smoker alveolar macrophages to inactivate α1AT as an inhibitor of neutrophil elastase. When alveolar macrophages were incubated with α1AT in the in vitro system designed to reproduce the microenvironment of ELF, very different effects were observed between smokers' and nonsmokers' alveolar macrophages on the antielastase function of the α1AT present. The nonsmokers' macrophages caused almost no loss of function of the α1AT as an inhibitor of neutrophil elastase (Fig. 2). In contrast, smoker alveolar macrophages caused a significant loss of the antielastase function of α1AT. The loss of antielastase function was present at all elastase-α1AT incubation intervals measured. Lack in increase in antielastase function with time suggests that a significant proportion of the population of α1AT molecules were rendered completely impotent as inhibitors of neutrophil elastase. Strikingly, the ability of smokers' macro-

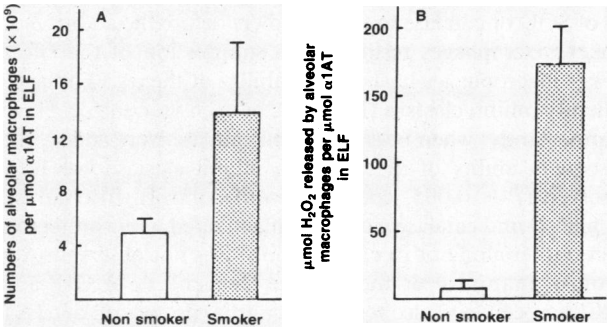

Figure 1. Relationship of alveolar macrophages and α1AT in the epithelial lining fluid of the lower respiratory tract of nonsmokers and smokers. (*A*) Ratio of number of alveolar macrophages recovered by lavage to amount of α1AT in the same lavage sample. (*B*) Ratio of amount of H_2O_2 spontaneously released by alveolar macrophages/h^{-1} to the amount of α1AT in ELF.

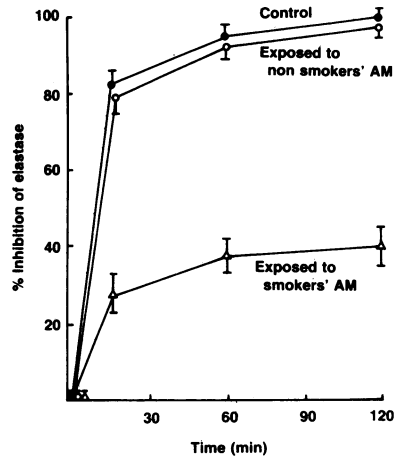

Figure 2. Effect of low-molecular weight mediators spontaneously released by alveolar macrophages of nonsmokers and smokers to suppress the ability of α1AT to inhibit neutrophil elastase. α1AT (1 μmol) was exposed to alveolar macrophages (10^6/ml; 4.65×10^9 macrophages/μmol α1AT) in chambers for 18 h, 37°. Dialysis tubing (mol wt cutoff, 12–14 kD) separated the macrophages from the α1AT. The α1AT was recovered from the chamber and evaluated for its ability to inhibit neutrophil elastase over time (2 nM elastase, 0–8 nM α1AT). Shown is the elastase inhibitory capacity of α1AT incubated in the chamber without alveolar macrophages on the other side of the membrane (●); α1AT exposed to alveolar macrophages of nonsmokers (○); and α1AT exposed to alveolar macrophages of smokers (△).

phages to inactivate α1AT occurred despite that fact that conditions within the assay mimicked those within the nonsmokers' alveolus rather than those of the smoker alveolus. In this regard, when the number of smokers' alveolar macrophages was increased beyond the ratio of alveolar macrophages to α1AT ELF found in nonsmokers, the loss of antineutrophil activity was even greater, with the loss of activity proportional to numbers of macrophages in the chamber (data not shown). When put in the context of the microenvironment of the ELF of the smoker, where the ratio of macrophages per micromole α1AT ELF is threefold greater than that used in this study, the observed effect upon α1AT (Fig. 2) probably represents the minimum reduction of α1AT antielastase activity ongoing in the lower respiratory tract of smokers.

Effect of antioxidants on ability of mediators released by smoker alveolar macrophages to interfere with α1AT antielastase function. When antioxidants were added to the incubations containing smoker alveolar macrophages and α1AT, the suppression of α1AT antielastase function caused by the smoker macrophages was not observed. In this regard, addition of SOD or catalase to the chambers before the addition of smoker macrophages resulted in a suppression of the effect smoker macrophages have on the ability of the α1AT to subsequently inhibit elastase ($P < 0.01$ for each vs. control; Table I). Importantly, when both SOD and catalase were added, the subsequent ability of the α1AT to inhibit elastase was fully preserved ($P < 0.005$, compared with control). In contrast, when SOD and catalase were heat-inactivated, their protective effect (either alone or in combination) was not observed. Addition of mannitol or methionine (scavengers of $OH^{\cdot}$ and HOCl, respectively) also resulted in partial protection of α1AT antielastase capacity (mannitol, 64 + 4, $P < 0.05$ vs. control; methionine, 63 + 7, $P < 0.05$ vs. control). These findings indicate that low-molecular weight oxidants were the mediators responsible for the observed loss of the elastase inhibitory function of α1AT after its incubation with smokers' alveolar macrophages. This is consistent with the observation that

Table I. Effect of Antioxidants on Ability of Low-Molecular Weight Mediators Spontaneously Released by Alveolar Macrophages of Cigarette Smokers to Suppress the Ability of α1-Antitrypsin to Inhibit Elastase

Condition	Inhibition of Elastase*
	%
α1AT exposed to smoker macrophages	27±12
+ in the presence of SOD‡	68±9
+ in the presence of catalase§	71±2
+ in the presence of SOD + catalase	98±2
+ in the presence of mannitol‖	64±4
+ in the presence of methionine¶	63±7

* All assays contained 8 nM active porcine pancreatic elastase and 0–20 nM α1AT; 100% inhibition = all of the elastase was inhibited. ‡400 U/ml. Heat-inactivated SOD gave no protection (26±11% inhibition of elastase). §1,000 U/ml. Heat-inactivated catalase gave no protection (30±15% inhibition of elastase). ‖1 mM. ¶1 mM.

smoker macrophages release exaggerated amounts of H_2O_2, $O_2^{\cdot}$ and $OH^{\cdot}$, and can utilize myeloperoxidase (either their own endogenous enzyme or myeloperoxidase scavenged from neutrophils) to generate the strong oxidant HOCl.

The met^{358} active site of α1AT as a target for smoker macrophages. The ability to produce recombinant forms of α1AT that are identical except for a single amino acid substitution at the active inhibitory site provides a means to confirm that at least part of the mechanism by which alveolar macrophages inactive α1AT as an inhibitor of elastase is by oxidizing the met^{358} residue at the site that is central to the elastase-inhibitory function of the α1AT molecule. In this regard, cigarette smokers' alveolar macrophages caused a nearly total loss of the ability of met^{358} rα1AT to inhibit neutrophil elastase (Fig. 3 *A*). In contrast, the ability of val^{358} rα1AT, to inhibit neutrophil elastase was not significantly affected by exposure to cigarette smokers' macrophages (Fig. 3 *B*). On the average, when compared with control met^{358} rα1AT not exposed to macrophages, the met^{358} rα1AT exposed to smoker macrophages was only 14±6% as active (Fig. 3 *C*). In contrast, val^{358} rα1AT retained 84±8% of its activity ($P < 0.005$). Because the only difference between met^{358} rα1AT and val^{358} rα1AT is the substitution of valine for methionine at the active site of the α1AT molecule, this differential effect strongly suggests that the mechanism of suppression of α1AT antielastase activity by smokers' macrophages is associated with the action of oxidants upon the active site methionine residue.

Discussion

The finding that cigarette smokers' alveolar macrophages spontaneously are releasing sufficient amounts of oxidants to modulate the neutrophil elastase-inhibitory function of α1AT provides insight into the mechanisms through which cigarette smoking is linked to a markedly increased risk for the development of emphysema. In cigarette smokers, alveolar macrophages are present in the lower respiratory tract in numbers severalfold greater than in nonsmokers (17, 29, 30). Characteristically they accumulate in terminal bronchioles, where they represent an early pathologic lesion in asymptomatic cig-

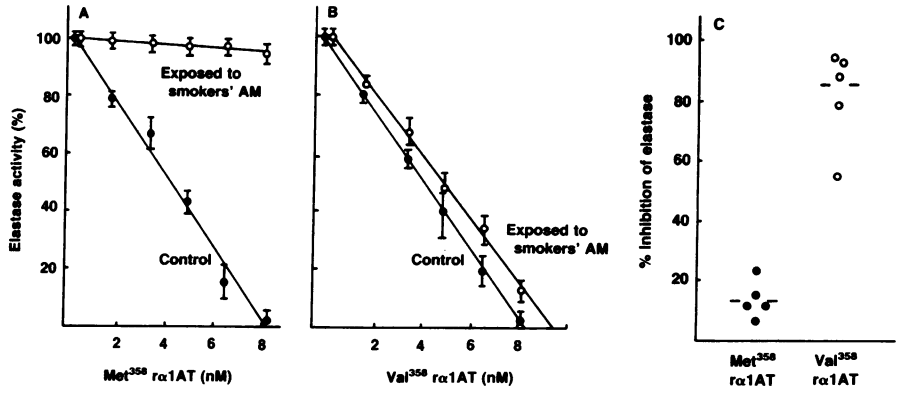

Figure 3. Effect of low-molecular weight mediators spontaneously released by alveolar macrophages of cigarette smokers on the ability of recombinant forms of α1AT to inhibit neutrophil elastase. Recombinant α1AT (1 μM) was separated from smokers' alveolar macrophages as in Fig. 2. Control α1AT was placed in identical chambers but not exposed to alveolar macrophages. After 18 h, 37°, α1AT was retrieved and tested for its ability to inhibit neutrophil elastase (8 nM) over a 1-h incubation period. (*A*) Recombinant met^{358} α1AT. Shown is an example from one individual; data are presented as percent activity of the neutrophil elastase remaining. (*B*) Recombinant val^{358} α1AT. Shown are data from one individual presented as in panel *A*. (*C*) Comparison of proportion of recombinant met^{358} α1AT and recombinant val^{358} α1AT capable of inhibiting neutrophil elastase after exposure to low-molecular weight products spontaneously released by alveolar macrophages of cigarette smokers. Recombinant met^{358} α1AT and val^{358} α1AT were exposed to smokers' alveolar macrophages in chambers as described in panels *A* and *B*. The incubation was continued for 18 h at 37°, after which the elastase-inhibitory capacity of the recombinant α1AT was determined. Each point represents a single individual.

arette smokers (50). Alveolar macrophages, by virtue of their increased numbers and exaggerated production of oxidants (29, 32, 33), are ideal candidates to effect the functional integrity of the antielastase screen of the lower respiratory tract. In this regard, in the context of the present study, it is reasonable to conceptualize that macrophages create local areas within the alveolus in which there is deficient antineutrophil elastase protection due to oxidation of α1AT. In this scenario, lack of antielastase screen makes these areas of the lung vulnerable to elastase released by neutrophils or by macrophages that have ingested neutrophil elastase (51, 52). The result would be development of localized tissue destruction that ultimately may become the clinical disorder emphysema.

Oxidation of α1AT by smokers' alveolar macrophages is also consistent with the study by Carp et al. (23) that observed oxidized α1AT in bronchoalveolar lavage fluid obtained from smokers' lungs. These observations are consistent with the knowledge that oxidized α1AT has a reduced association rate constant for neutrophil elastase (11) and that smoker lower respiratory tract α1AT has a lower association rate constant for neutrophil elastase than does nonsmoker lower respiratory tract α1AT (22). However, the demonstration that α1AT can be oxidized by smokers' macrophages does not necessarily imply that a significant proportion of the α1AT in all regions within the lower respiratory tract is oxidized. A more reasonable conceptualization based upon this model is that some α1AT is inactivated by oxidants released by the macrophages in areas of macrophage accumulation. This process may not be occurring to the same degree everywhere within the lung, due to local differences in macrophage concentration, availability of antioxidants, transudation of plasma, or other factors that could have the effect of decreasing the local intensity of alveolar macrophage exposure to α1AT. Furthermore, because cigarette smoke itself can oxidize α1AT, the extent, type, and technique of smoking may also influence the status of α1AT in different areas of the lower respiratory tract (19, 24). Hence, α1AT recovered by bronchoalveolar lavage may contain active and inactive α1AT in varying proportions. This heterogeneity may explain, at least in part, why some studies have shown that the population of α1AT molecules recovered from the lower respiratory tract of cigarette smokers is relatively unable to inhibit pancreatic elastase compared with that recovered from nonsmokers (13, 23, 24), whereas other studies have not shown such a difference (25, 26).

The fact that alveolar macrophages in the lower respiratory tract of cigarette smokers are capable of modifying α1AT in the local milieu by oxidant mechanisms has implications for future approaches to investigating therapeutic strategies for preventing emphysema associated with cigarette smoking. First, because alveolar macrophages are replaced slowly (53, 54), this may explain why some individuals continue to develop lung destruction even after discontinuing cigarette smoking. Therefore, therapeutic modalities directed at suppressing the exaggerated oxidant release by the macrophages may be useful. Second, antioxidants may provide an important means of preserving the antielastase screen of the lower respiratory tract. Finally, while there are theoretical dangers in providing a "super" form of α1AT that cannot be inactivated, the val^{358} form of recombinant α1AT may be one means to protect the lower respiratory tract against a burden of neutrophil elastase when there is a concomitant burden of oxidants such as those released by alveolar macrophages of cigarette smokers.

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