Neutrophil Accumulation in the Lung in α1-Antitrypsin Deficiency

Spontaneous Release of Leukotriene B_4 by Alveolar Macrophages

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Abstract

The emphysema of α1-antitrypsin (α1AT) deficiency is conceptualized to result from insufficient α1AT allowing neutrophil elastase to destroy lung parenchyma. In addition to the deficiency of α1AT in these individuals resulting from mutations in the α1AT gene, it is recognized that, for unknown reasons, there are also increased numbers of neutrophils in their lungs compared with normal individuals. With the knowledge that alveolar macrophages have surface receptors for neutrophil elastase, we hypothesized that the neutrophil accumulation in the lower respiratory tract in α1AT deficiency may result, in part, from release of neutrophil chemotactic activity by alveolar macrophages as they bind uninhibited neutrophil elastase. Consistent with this hypothesis, α1AT-deficient alveolar macrophages spontaneously released nearly threefold more neutrophil chemotactic activity than normal alveolar macrophages. Analysis of α1AT-deficient macrophage supernates by reverse-phase HPLC, molecular sieve chromatography, radioimmunoassay, and absorption with anti-LTB_4 antibody revealed that the majority of the chemotactic activity was leukotriene B_4 (LTB_4), a mediator absent from normal macrophage supernates. Consistent with this hypothesis, incubation of normal macrophages with human neutrophil elastase resulted in the release of the same neutrophil chemotactic mediator. Furthermore, purified human α1AT was able to prevent the neutrophil elastase from stimulating the macrophages to release the chemotactic factor. Together, these findings suggest that the absence of a normal antineutrophil elastase screen in the lower respiratory tract permits free neutrophil elastase to bind to alveolar macrophages, resulting in the release of LTB_4, a process which attracts neutrophils to the alveoli of α1AT deficient individuals, thus accelerating the lung destruction that characterizes this disorder. (*J. Clin. Invest.* 1991. 88:891–897.) Key words: emphysema • inflammation • bronchoalveolar lavage • neutrophil elastase antiprotease

Introduction

α1-Antitrypsin (α1AT)[1] deficiency is an autosomal hereditary disorder characterized by reduced serum levels of α1AT, an increased risk for the development of emphysema, and a lesser, but definite risk for hepatitis and cirrhosis (1–6). In the context of the knowledge that α1AT provides almost all of the protective screen of the lower respiratory tract against the proteolytic enzyme neutrophil elastase (NE), the emphysema of α1AT deficiency is understood to result from insufficient levels of α1AT in the lower respiratory tract to effectively inhibit the burden of NE in the lungs (7–9). Because NE is capable of degrading the connective tissue matrix of the alveolar walls, the chronic burden of free, uninhibited elastase is associated with alveolar wall destruction, i.e., emphysema (1, 3, 5, 8–10).

While the deficiency of α1AT in this disorder is understood to result from various mutations in five α1AT coding exons (3, 4), the destruction of the alveolar walls could not occur without the chronic presence of neutrophils within the lung. Interestingly, in previous studies of α1AT in the epithelial lining fluid of the lower respiratory tract of nonsmoking α1AT deficient individuals, we have observed that there are more neutrophils than observed in normals (11, 12). On the basis of these observations, the present study is directed at evaluating the hypothesis that neutrophils accumulate in the alveolar structures of these individuals at least in part because they are attracted by chemotactic signals released by alveolar macrophages. This hypothesis is based on three concepts, including: (*a*) alveolar macrophages are capable, when activated by surface stimuli, of releasing chemotactic mediators for neutrophils (13–19); (*b*) alveolar macrophages have surface receptors for neutrophil elastase (20–22); and (*c*) individuals with α1AT deficiency have insufficient α1AT in the lower respiratory tract to inhibit the burden of NE in the local milieu and thus likely have free NE in the alveolar structures (7, 23). Putting these concepts together, they lead to a scenario of amplification of normal inflammatory processes, in which free NE resulting from insufficient α1AT binds to the NE receptors in alveolar macrophages, causing the macrophages to release mediators with neutrophil chemotactic activity, thus causing an increased number of neutrophils to accumulate in the alveolar structures. Consequently this process would further add to other likely causes of altered alveolar macrophage function in α1AT-deficient patients, including cigarette smoking, airway infection, and the presence of matrix degradation products.

Methods

Study population. The study population consisted of 14 individuals with the Z homozygous form of α1AT deficiency, verified by serum α1AT levels, isoelectric focusing of serum, and family analysis (24). There were five females and nine males with an average age of 40±5 yr (all data presented as mean±standard error of the mean, and all statistical comparisons were made using the two-tailed Student's *t* test). The average serum α1AT level was 4.6 μM (normal 20–48 μM; based on a true laboratory standard; see reference 23 for details). There were six lifelong nonsmokers, and eight former cigarette smokers; the former smokers had stopped an average of 3.5±2.6 yr before the study, with a

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1. *Abbreviations used in this paper:* α1AT, α1-antitrypsin; LTB_4, leukotriene B_4; NE, neutrophil elastase.

minimum of 1 yr previously. All individuals had clinical evidence of emphysema based on physical examination, chest x-ray, xenon-133 ventilation scans, technetium-99m-labeled macroaggregated albumin scans and lung function studies (25) (vital capacity 74±15% predicted, forced expiratory volume in 1 s 59±19% predicted, and diffusing capacity for carbon monoxide 65±15% predicted [corrected for hemoglobin and volume]).

For comparison, a population of 11 normal individuals included seven men and four women with an average age of 29±5 yr. All were nonsmokers and none had evidence of lung disease by history, physical examination, chest x-ray, and pulmonary function tests. None were taking any medications. Serum α1AT phenotyping demonstrated all were of the MM phenotype (i.e., various combinations of the normal M1(Val^{213}), M1(Ala^{213}), M2, and M3 alleles) (3, 4). As a group, the normals had a serum α1AT level of 39±4 μM.

Quantification of neutrophils in epithelial lining fluid. Bronchoalveolar lavage and cell analysis was carried out in each individual as previously described (26). The volume of epithelial lining fluid (ELF) recovered was quantified by the urea method and the total number of neutrophils recovered was calculated and expressed per unit volume of ELF recovered (27).

Release of neutrophil chemotactic activity by alveolar macrophages. Alveolar macrophages obtained by bronchoalveolar lavage were incubated (10^6 cells/ml, 37°) in RPMI-1640 media (Whittaker M. A. Bioproducts, Walkersville, MD) in sterile 24-well culture plates (Falcon Labware, Oxnard, CA). After a 1-h incubation period to permit adherence, the cells were washed three times with iced RPMI-1640, after which additional RPMI-1640 (1 ml) was added to each well. After incubation (3 h, 37°), the supernate was evaluated for neutrophil chemotactic activity (see below). The viability of macrophages at the end of the incubation period was > 90% in all cases, as determined by trypan blue exclusion (14).

Neutrophil chemotactic activity was quantified using a 48-well microchemotaxis chamber (Neuro Probe, Inc., Cabin John, MD) and 3-μM pore size filters (Nuclepore, Pleasanton, CA) (28). Macrophage supernate or RPMI-1640 media alone (as a negative control) was placed in the lower portion of the chamber, and 10^5 human neutrophils (prepared from a single donor by Plasmagel [Cellular Products, Buffalo, NY] sedimentation and hypotonic lysis of residual erythrocytes, neutrophil purity > 90% and viability > 95% as assessed by trypan blue exclusion [29]) in RPMI-1640 were added to the upper chamber. After incubation (30 min, 37°), the filters were removed, wiped, and stained with Diff Quik (American Scientific Products, McGraw Park, IL) and the number of neutrophils per 10 randomly selected high-power microscope fields which had reached the lower surface of the micropore filter was determined. Each supernate was assayed in triplicate, for a total number of 30 high-power fields (hpf), and the neutrophil chemotactic activity of each supernate determined as the mean number of cells counted per 10 hpf. The neutrophil chemotactic activity was expressed as the mean number of cells counted per 10 hpf of a sample supernate, minus the mean number of cells responding to a simultaneously assayed control consisting of RPMI-1640 media alone.

To insure that the migration of neutrophils across the membrane in response to macrophage supernates was due to directed migration (chemotaxis) and not to increased random migration (chemokinesis), a checkerboard analysis was performed in which varying amounts of alveolar macrophage supernate were added to both the upper and lower compartments of the chemotaxis chamber.

Identification of LTB_4 in alveolar macrophage supernates. Reverse-phase HPLC was performed in the manner of Kouzan et al. (30) with minor modifications. Supernates from 2×10^6 alveolar macrophages in RPMI-1640, were diluted with 4 vol ice-cold HPLC grade ethanol (Aldrich Chemical Co., Milwaukee, WI), vortexed, and evaporated to dryness at 37° under a stream of dry nitrogen. The residual material was dissolved in 250 μl 30% methanol and applied to a C18 Ultrasphere column (4.6 × 25 cm, Beckman Instruments, Inc., Fullerton, CA). Successive isocratic elution steps were performed at a flow rate of 1 ml/min using two solvent mixtures: solvent A, 90% water/10% methanol/0.08% acetic acid; solvent B, 100% methanol. Step 1 (0–27 min), 45% solvent B; step 2 (27–70 min), 67% solvent B; step 3 (70–100 min), 100% solvent B. Identification of LTB_4 in supernate assays was established by coelution with a LTB_4 standard (provided by Rokach, J., Merck Frosst, Canada) with the column eluate monitored at 280 nm. Separate chromatographic analyses were performed on five ZZ and five MM individuals.

Direct measurement of the concentration of LTB_4 in alveolar macrophage supernates was performed using a radioimmunoassay specific for LTB_4 with a lower limit of detectability of 0.08 ng/ml (31, 32). Alveolar macrophages (2×10^6) were cultured as described above, and the supernates directly analyzed without purification for the presence of immunoreactive LTB_4.

Determination that LTB_4 is responsible for the chemotactic activity of alveolar macrophage supernates of Z homozygotes. To demonstrate that LTB_4 was responsible for the majority of the neutrophil chemotactic activity present in supernates from ZZ alveolar macrophages, molecular sieve chromatographic analysis was carried out and the peak neutrophil chemotactic activity evaluated by reverse phase HPLC in supernates of three ZZ individuals. Supernate (500 μl) from 3×10^6 ZZ alveolar macrophages cultured in HBSS was applied to Superose 12 and Superose 6 molecular sieves (Pharmacia Fine Chemicals, Piscataway, NJ) connected in series. The entire column eluate was collected in 1-ml fractions, and each fraction was separately assayed for its chemotactic activity in filter chambers as described above. The peak fractions demonstrating significant chemotactic activity were collected and subsequently analyzed by reverse-phase HPLC as described above.

To positively identify the chemotactic factor induced by elastase, supernates (n = 3) from elastase exposed MM alveolar macrophages and alveolar macrophages from ZZ individuals (n = 3) were individually applied to an affinity chromatography column containing rabbit anti-human LTB_4 antibody (supplied by Ford-Hutchinson, A., Merck Frosst, Canada). As a negative control a column containing rabbit anti-human lysozyme was used. Before and after chromatography, the supernates were evaluated for chemotactic activity in filter chambers as described above.

Assessment of the ability of neutrophil elastase to stimulate the release of LTB_4 by alveolar macrophages. To evaluate the concept that neutrophil elastase might be responsible for the stimulation of alveolar macrophages to release neutrophil chemotactic activity, purified human neutrophil elastase (100 nM), in RPMI-1640 was added to normal (phenotype MM) alveolar macrophages (30 min, 37°) and the supernatants evaluated for neutrophil chemotactic activity as described above.

To determine whether α1AT had a modulating effect upon the macrophage elaboration of chemotactic activity in response to neutrophil elastase, α1AT purified from MM human plasma (33) was incubated at concentrations ranging from 50 to 500 nM with 100 nM neutrophil elastase before addition to the MM alveolar macrophages. After a 30-min incubation, the macrophage supernates were collected and assayed for neutrophil chemotactic activity.

Results

Neutrophils in the lower respiratory tract in α1-antitrypsin deficiency. Quantification of the lung neutrophil burden by bronchoalveolar lavage demonstrated that ZZ individuals had a greater number of neutrophils in their alveolar epithelial lining fluid than did normal MM individuals. In this regard, ZZ individuals contained almost threefold more neutrophils in ELF than normals ($P < 0.05$) (Fig. 1). In contrast the numbers of all other cell types (macrophages, lymphocytes, and eosinophils) was similar in both groups, ($P > 0.5$ all comparisons). Importantly, the increased number of neutrophils was present without evidence of infection, or overt inflammation in the form of hyperemia of the airways or increased bronchial mucous. Furthermore, there was no significantly increased number of bronchial cells in the ZZ lavage fluid compared to normal.

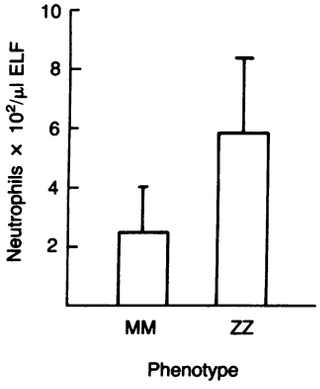

Figure 1. Characterization of the lung neutrophil burden in epithelial lining fluid from MM and ZZ individuals. Shown is the number of neutrophils per microliter epithelial lining fluid (ELF) recovered by bronchoalveolar lavage of nonsmoking MM and ZZ individuals.

Spontaneous release of neutrophil chemotactic activity. Consistent with the observed increase in numbers of neutrophils in ELF of the ZZ individuals, when alveolar macrophages recovered from these individuals were cultured in vitro, they spontaneously released significantly greater amounts of neutrophil chemotactic activity than MM alveolar macrophages, ($P < 0.01$) (Fig. 2). Checkerboard analysis of ZZ macrophage supernate demonstrated that the neutrophil migration was due to true chemotactic activity, not chemokinesis (Table I). Furthermore, the spontaneous release of neutrophil chemotactic activity by ZZ alveolar macrophages equaled the neutrophil chemotactic factor released by MM alveolar macrophages stimulated in vitro by zymosan ($P > 0.5$ compared to spontaneous release by ZZ macrophages).

Analysis of alveolar macrophage supernates for LTB_4. In the context of the knowledge that LTB_4 is the major neutrophil chemotactic factor released by human alveolar macrophages, the supernates of ZZ and MM alveolar macrophages cultured in vitro were analyzed for the presence of LTB_4. Using a C18 reverse-phase column, we identified an LTB_4 standard by its elution time (Fig. 3 *A*). Consistent with the comparative lack of neutrophil chemotactic activity present in the supernates of MM alveolar macrophages, no LTB_4 was evident on the chromatogram of the MM alveolar macrophage supernates (Fig. 3 *B*). In contrast, supernates of ZZ alveolar macrophages contained LTB_4, as determined by the presence of a chromatographic peak eluting at the same time as the LTB_4 standard (Fig. 3 *C*). Consistent with the chromatographic analysis, quantitative analysis of LTB_4 production by MM and ZZ alveolar macrophages by radioimmunoassay also demonstrated that the ZZ alveolar macrophages spontaneously released exaggerated amounts of LTB_4 compared with MM alveolar macrophages. In this regard, the ZZ alveolar macrophages released threefold more LTB_4 than unstimulated MM macrophages ($P < 0.01$) (Fig. 4), an observation consistent with the relative amount of neutrophil chemotactic activity observed in the supernates of the ZZ and MM macrophages (Fig. 2).

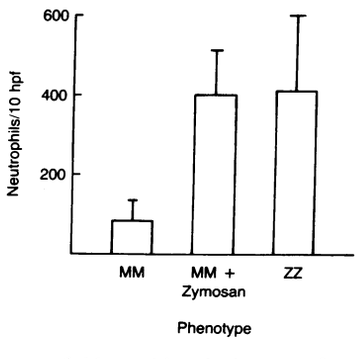

Figure 2. Spontaneous release of neutrophil chemotactic activity by alveolar macrophages from MM and ZZ individuals. For comparison, also shown is the neutrophil chemotactic activity released by MM alveolar macrophages stimulated in vitro with zymosan (100 μg/ml). Neutrophil chemotactic activity is expressed as the number of neutrophils per 10 high-power fields reaching the lower surface of a micropore filter.

Table I. Checkerboard Analysis of Neutrophil Chemotactic Activity Spontaneously Released by Alveolar Macrophages of Individuals with the Z Homozygous Form of α1-Antitrypsin Deficiency

Relative concentration of supernate in lower chamber	Relative concentration of AM supernate in upper chamber		
	0%	50%	100%
%	%	%	%
0	80±11	89±14	66±9
50	29±24	202±28	169±21
100	541±29	312±31	173±26

Alveolar macrophages recovered from the lower respiratory tract of individuals with α1AT deficiency were incubated (3 h, 37°) and the supernates evaluated for neutrophil chemotactic activity in a chemotactic chamber (see Methods). The data is presented as the mean number of cells per 10 high-power fields reaching the lower surface of a micropore filter.

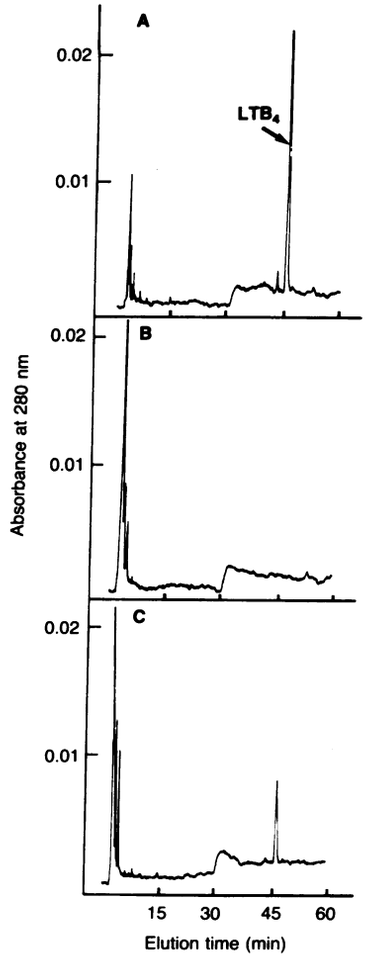

Figure 3. C18 reverse-phase HPLC analysis of supernates form MM and ZZ alveolar macrophages. (*A*) LTB_4 standard. (*B*) Supernate generated by 2×10^6 unstimulated MM alveolar macrophages. (*C*) Supernate from 2×10^6 unstimulated ZZ alveolar macrophages. Note material with an elution time identical to the LTB_4 standard. *Ordinate,* absorbance at 280 nm; *abscissa,* elution time.

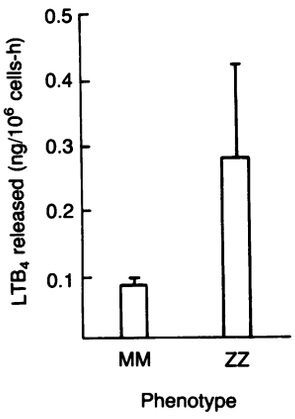

Figure 4. Amount of LTB_4 spontaneously released by MM (n = 11) and ZZ (n = 11) alveolar macrophages. Quantification was performed by radioimmunoassay; the data is expressed as the amount of LTB_4 released by 2×10^5 alveolar macrophages form each individual over a 3-h period. The difference is significant ($P < 0.01$).

Demonstration that LTB_4 is the neutrophil chemotactic factor in ZZ alveolar macrophage supernates. To directly demonstrate that LTB_4 was the major chemotactic mediator responsible for the neutrophil chemotactic factor activity found in ZZ alveolar macrophage supernates, combined molecular sieve and reverse-phase HPLC was performed on supernates of unstimulated ZZ macrophages cultured in vitro. When fractions of ZZ macrophage supernate that had been separated according to size on a molecular sieve were analyzed for their neutrophil chemotactic activity, the major peak of chemotactic activity occurred in the size range between 1,000 and 200 D, a size consistent with that of LTB_4 (336 D) (Fig. 5 *A*). When these pooled low-molecular weight fractions containing neutrophil chemotactic activity were then analyzed by C18 reverse-phase chromatography, LTB_4 was observed (Fig. 5 *B*).

Antibody absorption studies definitively identified the chemotactic factor as LTB_4. When supernates from elastase exposed MM alveolar macrophages were passed through an affinity chromotography column containing antibody against LTB_4 the neutrophil chemotactic activity of the supernates was lost. No such loss of chemotactic activity was seen following supernate exposure to a column containing antibody against human lysozyme (not shown). Similarly, passage of supernates from PiZZ alveolar macrophages through the anti-LTB_4 affinity column resulted in loss of the majority of the neutrophil chemotactic activity (Fig. 6).

Neutrophil elastase stimulation of neutrophil chemotactic factor release by MM alveolar macrophages. Consistent with the possibility that it is uninhibited neutrophil elastase in the lower respiratory tract that provides the activation signal to the alveolar macrophages, when MM alveolar macrophages were incubated in vitro with neutrophil elastase, neutrophil chemotactic factor was released in amounts similar to that observed after zymosan incubation (Fig. 7 *A*). Furthermore, purified human α1AT was able to modulate the neutrophil elastase stimulated release of neutrophil chemotactic factor from MM alveolar macrophages in a dose-dependent manner. In this regard, incubation of α1AT with neutrophil elastase before its addition to the macrophages resulted in a nearly complete reduction in the stimulation of neutrophil chemotactic factor release by MM alveolar macrophages (Fig. 7 *B*). The dose-dependent ability of α1AT to suppress the effect of elastase on the MM macrophages is consistent with the known function of α1AT to bind to and inactivate neutrophil elastase.

Discussion

The present study suggests that one process which may contribute to the accelerated lung destruction that characterizes α1AT deficiency is a chronically increased neutrophil burden in the lower respiratory tract caused by alveolar macrophages releasing neutrophil chemotactic factor LTB_4. A likely mechanism which explains this increased release of LTB_4 is the binding of free neutrophil elastase by ZZ alveolar macrophages. Consistent with this hypothesis, the ability of elastase to mediate the release of LTB_4 does not occur if sufficient α1AT is present. These observations suggest a pathologic scenario in which the deficiency of α1AT in the lungs of ZZ individuals results in the presence of free elastase, which then interacts with surface re-

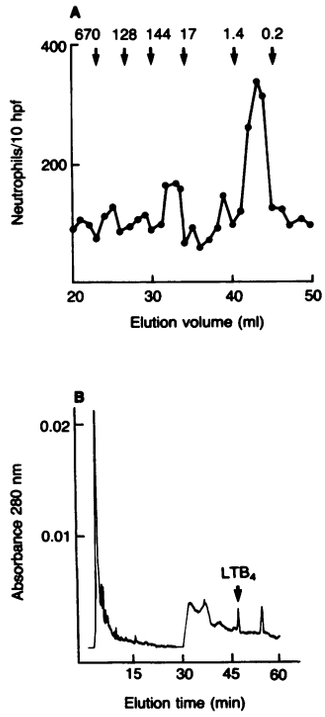

Figure 5. Correlation of the neutrophil chemotactic activity in the supernates of ZZ alveolar macrophages with LTB_4 spontaneously released by the same alveolar macrophages. (*A*) Molecular sieve chromatographic analysis of supernate of unstimulated ZZ alveolar macrophages demonstrating the neutrophil chemotactic activity of fractions separated according to their relative molecular weights. Neutrophil chemotactic activity is expressed on the ordinate as the number of neutrophils per 10 high-power fields reaching the lower surface of a micropore filter. (*B*) C18 reverse-phase HPLC analysis of the fractions demonstrating neutrophil chemotactic activity (panel *A* fractions 41–45). The position of elution of an LTB_4 standard is indicated. Numbers at top indicate molecular weight.

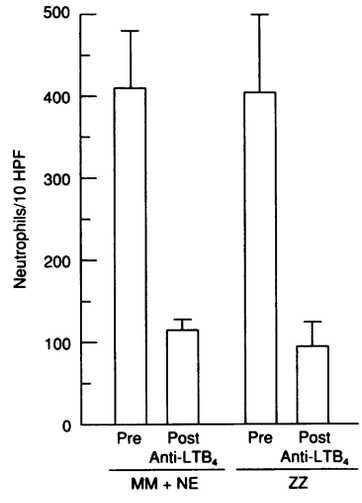

Figure 6. Identification of neutrophil chemotactic factor activity in supernates of neutrophil elastase (100 nM) exposed MM alveolar macrophages and supernates of ZZ alveolar macrophage as LTB_4. Macrophage supernates were assayed for neutrophil chemotactic activity before and after application to an affinity column containing anti-LTB_4 antibody. Neutrophil chemotactic activity is expressed as the number of neutrophils per 10 high-power fields reaching the lower surface of a micropore filter.

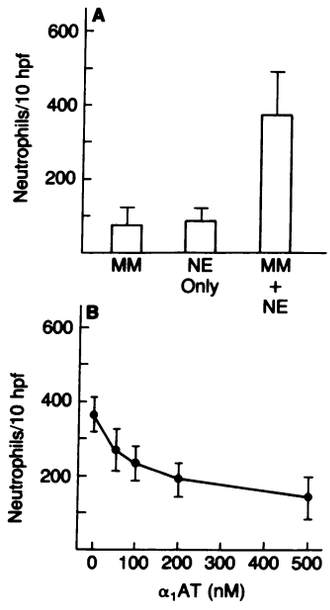

Figure 7. Demonstration that neutrophil elastase can stimulate normal MM alveolar macrophages to release neutrophil chemotactic factor activity. (*A*) Neutrophil chemotactic activity spontaneously released into the supernate by MM alveolar macrophages after exposure of the macrophages to NE (100 nM). NE by itself had no chemotactic activity. (*B*) Ability of α1-antitrypsin (α1AT) to prevent neutrophil elastase from stimulating normal MM alveolar macrophages to release neutrophil chemotactic activity. Shown is the neutrophil chemotactic activity of supernates of MM alveolar macrophages exposed to neutrophil elastase which had been preincubated with various concentrations of α1-antitrypsin. Neutrophil chemotaxis is expressed as the number of neutrophils per 10 high-power fields reaching the lower surface of a micropore filter.

ceptors on alveolar macrophages, stimulating the release of LTB_4 by the macrophages and consequently the recruitment of still more neutrophils with their burden of elastase, thus establishing a cycle characterized by a chronically increased lower respiratory tract proteolytic burden. In this context, the imbalance between neutrophil elastase and α1AT in the lower respiratory tract of α1AT-deficient individuals is beyond that attributable to the α1AT deficiency itself, but is further increased by exaggeration of the neutrophil, and hence neutrophil elastase, burden.

Alveolar macrophage interaction with neutrophil elastase. Neutrophils are present in the lungs of normal individuals in small numbers. A variety of mediators have been identified which likely play a role in neutrophil recruitment into the lungs, including bacterial endotoxin, complement fragments, platelet-activating factor, interleukin-8, the bacterial equivalent of formyl-methionyl-leucyl-phenylalanine, other arachidonic acid metabolites, and one or more polypeptides (13, 16–19, 34–41). The central role of the alveolar macrophage as a mediator of neutrophil accumulation has been clearly documented (42–44). Importantly, as the initial cell to encounter foreign substances in the lower respiratory tract, the macrophage is capable of amplifying the defenses of the lower respiratory tract by recruiting neutrophils. It is recognized that LTB_4 is the predominant neutrophil chemotactic mediator released by alveolar macrophages (16–18, 39, 40, 42–44). In this regard, alveolar macrophages synthesize and release LTB_4 in response to a number of naturally occurring stimuli including immune complexes, IgE, bacteria, and other phagocytic stimuli such as asbestos (16–18, 35, 39, 44–47). Neutrophil elastase can now be added to the list of agents which result in release of LTB_4 by alveolar macrophages.

Although it was not the purpose of the present investigation to examine in detail the manner in which neutrophil elastase interacts with the alveolar macrophage to effect LTB_4 release, it is known that alveolar macrophages bind and internalize neutrophil elastase, mainly via a low-affinity, high-volume surface receptor (20–22). This receptor is not elastase-specific; cathepsin G and lactoferrin are also bound (21). Bound elastase is internalized by the cell via endocytosis, and is slowly degraded, but under some circumstances may be released in active form from the macrophage (22, 49). Binding of elastase by its receptor occurs in the absence of α2-macroglobulin and α1-antitrypsin, and is distinct from the macrophage uptake of α2-macroglobulin-protease complexes, which is thought to contribute to the removal of proteases from the extracellular space (20, 50). Interestingly, after exposure to a variety of neutral proteases including pancreatic elastase and collagenase, mouse peritoneal macrophages demonstrate increased prostaglandin synthesis and release of arachidonic acid metabolites (51). Therefore, the finding in the present study that alveolar macrophages specifically react to neutrophil elastase by the release of LTB_4 is consistent with what is known of alveolar macrophage binding of elastase via a surface receptor, and the subsequent activation of macrophage arachidonic acid metabolic pathways.

α1AT as a complex factor in modulating inflammation. By virtue of its principal physiologic role as an inhibitor of neutrophil elastase, α1AT plays an important role in modulating inflammation. By directly inhibiting elastase through the binding of elastase to its active site on a stressed, external loop with methionine[358] at its center, α1AT specifically acts to limit proteolytic activity of neutrophil elastase, thereby protecting bystander parenchymal tissues from proteolysis (52). The active site methionine[358] residue of α1AT can be oxidized by the section of endogenous or exogenously produced oxidants, resulting in the inactivation of α1AT as an effective antiprotease, with the result that elastase may act without the normal degree of restraint offered by active α1AT (53). This "switching off" of α1AT by oxidants may be beneficial in certain pathologic states such as infection, where elastolytic attack of a foreign organism is desirable, but in other circumstances, such as in cigarette smoking, adult respiratory distress syndrome, cystic fibrosis, or asbestos lung disease, the oxidative inactivation of α1AT likely leads to deleterious lung damage (54–60).

Another role played by α1AT in modulating inflammation is its ability to directly act as a chemoattractant after interaction with proteases. After proteolysis by mouse macrophage elastase, α1AT undergoes cleavage and structural reorganization, with the generation of a 4.2-kD fragment which is chemoattractive for neutrophils (61). A similar fragment can be generated after inactivation of human neutrophil elastase by α1AT, which also possesses neutrophil chemotactic activity (62). Therefore, α1AT contains latent intrinsic neutrophil chemoattractant properties which become manifest after interaction with its target protease, neutrophil elastase. This intrinsic chemotactic activity of α1AT unmasked after interaction with neutrophil elastase may also play a role in recruiting some of the neutrophils observed in the lungs of Z homozygotes, and may also be responsible for the observation in the present study that incubation of fivefold excess α1AT with elastase did not completely eliminate chemotactic activity in macrophage supernates, i.e., the residual chemotactic activity may have been due to release of chemotactic fragments from the α1AT molecule after its cleavage by elastase. The finding of the present study, that a deficiency of α1AT serves as the basis for an exaggerated, prolonged inflammatory response characterized by LTB_4-mediated neutrophil recruitment, represents a new concept of the role of α1AT in modulating inflammation. In this

regard, α1AT is not directly active as an inflammatory modulator, aside from its elastase-inhibitory properties. Rather, α1AT acts to interrupt a potential feedback cycle in which free elastase results in the recruitment of more neutrophils, hence more elastase, via the interaction of free elastase with macrophages which leads to elaboration of LTB_4. By inactivating free elastase, α1AT breaks the cycle; conversely deficiency of α1AT may permit amplification and perpetuation of the inflammatory reaction. As with oxidative "switching off" of α1AT, α1AT deficiency may be a "two-edged sword"—beneficial in the face of massive infection or other major threat to the organism when the deficiency is temporary and localized, but damaging in the chronic situation such as α1AT deficiency.

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